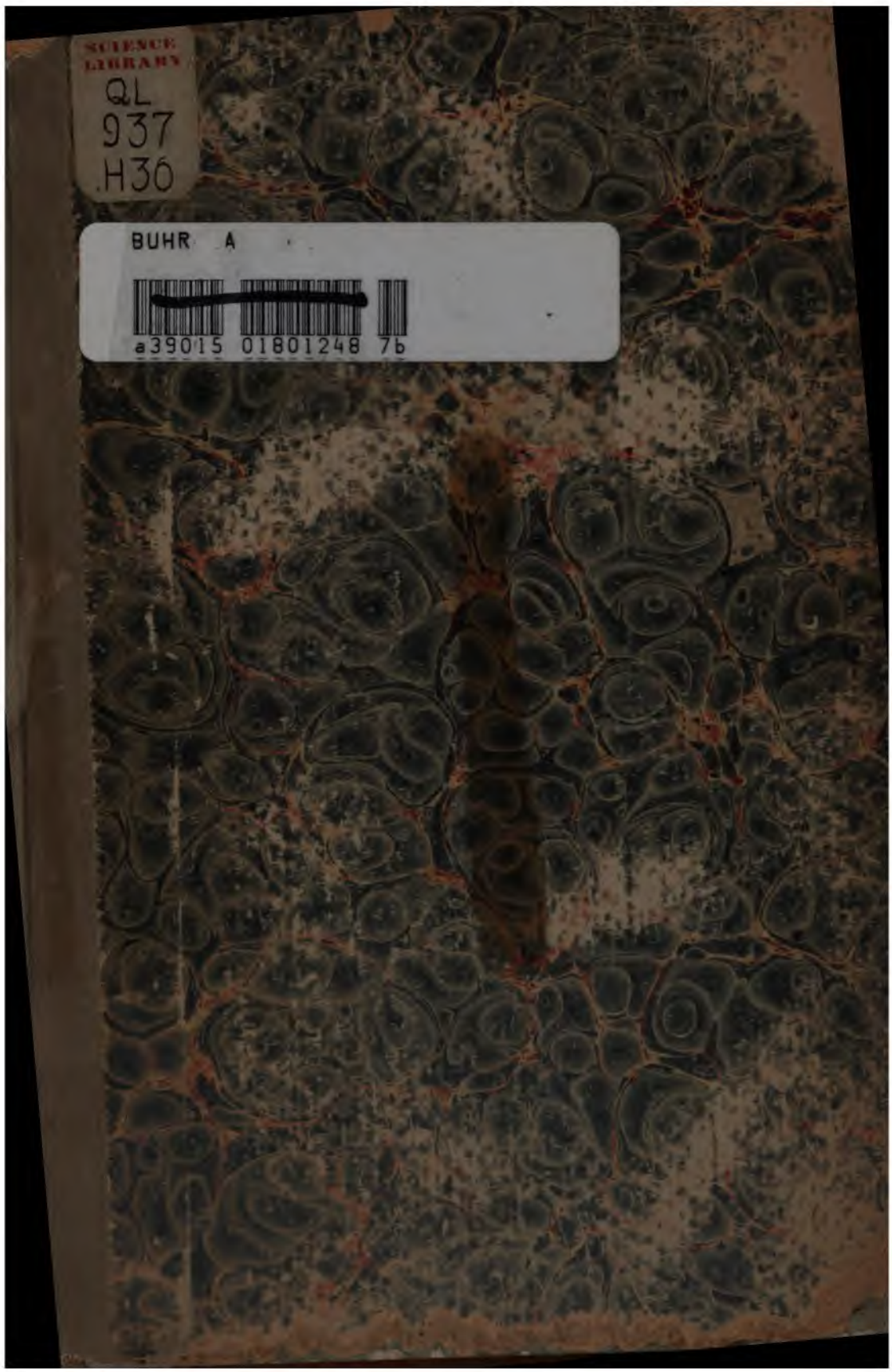


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STUDIES ON THE NERVOUS SYSTEM OF THE WHITE RAT AND FOETAL CAT

A DISSERTATION

SUBMITTED TO THE FACULTIES OF THE GRADUATE SCHOOLS OF ARTS, LITERATURE, AND SCIENCE, IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF NEUROLOGY)

BY
SHINKISHI HATAI

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THE FINER STRUCTURE OF THE SPINAL GANGLION CELLS IN THE WHITE RAT.

By SHINKISHI HATAI.

(From the Neurological Laboratory of the University of Chicago.)

With Plate I.

The spinal ganglion of the white rat contains two very different kinds of ganglion cells. One is larger in size and less compact in the structure of the cytoplasm than the other (Figs. 1, 2, 3) and also stains lightly with eosin and erythrosin. This kind has been the most studied by previous investigators and its structure is comparatively well known. The second kind is very compact in the structure of the cytoplasm (Figs. 4, 5), stains deeply and is not so well known. The two kinds of ganglion cells have been found in all vertebrates in which the spinal ganglia have been carefully studied.

The present paper deals with the structure and significance of the small spinal ganglion cells.

The observations recorded may conveniently be presented in the following order:

I. Technique.
II. Measurements of the cells in the spinal ganglion.
III. Internal structure of spinal ganglion cells.
IV. Review of growth changes in the spinal ganglion.
V. Summary.

I. TECHNIQUE.

The white rat was used for the present work. Since the nerve cells are rapidly altered after death and also undergo changes in old age—as shown by HODGE[1]—care was taken to use material from animals freshly killed, and mature, but not senescent.

White rats, between 100 and 150 grams in body weight, were employed. Specimens of this weight are in the prime of life.

After being chloroformed, the animal was dissected and the ganglia removed. These were preserved separately in the following fluids. The preserving fluids, as well as staining agents, which have been recommended by previous authors are marked by the author's name above the formula. Fluids which are not named were devised by the writer:

1. Carnoy's Fluid.

Alcohol . . .	60 cc
Chloroform . .	30 cc
Glacial acetic acid .	10 cc

2. Ewing's Fluid.

Mercuric chloride, sat. sol. in 5% Formalin.

3. Graf's Fluid.

Oxalic acid 80% .	4 vol.
Alcohol 95% .	3 vol.
Chromic acid 1% .	3 vol.

4. Lenhossèk's Fluid.

Corrosive sublimate, aq. sat. sol.

5. Gilson's Fluid.

Nitric acid of 46° strength .	78 cc
(This would be sp. gr. 1.456 or 80% nearly.)	
Glacial acetic acid . .	22 cc
Corrosive sublimate, aq. sat. sol.	50 cc
60% alcohol	500 cc
Distilled water . . .	440 cc

[1] HODGE—Changes in ganglion cells from birth to senile death. Observation on man and honey bees. *Journ. Physiology*, Vol. 17, '94.

Fluids devised by the author.

6.	Corrosive sublimate, Sat. sol. in formalin	30 cc
	Glacial acetic acid	50 cc
	Normal salt solution	15 cc
7.	Picric acid, sat. sol. in 10% formalin . .	60 cc
	Glacial acetic acid	20 cc
	Corrosive sublimate	20 cc

In the above tables, the fluids which were recommended by the previous authors were used to preserve the materials according to the directions given. The other fluids which were new were employed in the following manner:

The tissue should remain in these fluids for 6 to 12 hours, according to the size of the piece. Generally thin pieces give more satisfactory results. After fixing, the tissue should be transferred to running water where it should remain 4 to 5 hours. Then it should be transferred to weak alcohol, about 30%. The further procedure is the same as for ordinary paraffin embedding.

As staining agents, all of the following were used on each piece of tissue:

1. Toluidin blue, aq. sat. sol.
2. Thionin, aq. sat. sol.
3. NISSL'S fluid—as follows;

Methylene blue (powder) (methylene B. pat.) 1.3 grms.
White castile soap (Venice soap) . . 0.7 grms.
Distilled water . . . . . 332. cc.

For counter-staining:

1. Erythrosin 1% in 95% alcohol.
2. Eosin 1% in 80% alcohol.

As the clearing agents:

1. Xylol.
2. Cedar oil.

A comparison of sections prepared by the methods named above gave the following results. In each case, NISSL'S stainable substance and the non-stainable substance came out very beautifully. The materials fixed with GILSON'S fluid show greater shrinkage than those prepared by the other fluids. GRAF'S fluid

has a weak penetrating power. CARNOY'S mixture gives very satisfactory results. This is a very simple but most safe fluid for general use. The author's own mixture gave quite as satisfactory results as CARNOY'S. Especially for the study of differences between the axone and dendrites to be elsewhere described, the author's fluid is preferable to any of the other fluids. In this fluid, the neurosomes of the axis cylinder stain very deeply but the neurosomes of the dendrites stain only lightly. The cell-bodies keep quite a good shape and never swell, as in the case of CARNOY'S fluid.

But the author believes with EWING[1] who studied ganglion cells in the human nervous system, that "more important than the choice of any particular fixative is the care in handling the tissue, and the exclusive dependence upon thin pieces of tissue, 1 to 2 mm., thick, which can be rapidly penetrated by all agents."

For staining, the author most often used thionin and toluidin blue, but a methylene blue was occasionally used. Good results were obtained from each of the staining fluids.

A counter-staining fluid was always used in the present work. For this purpose, erythrosin and eosin were tried and erythrosin gave the more satisfactory results.

II. MEASUREMENTS OF THE CELLS IN THE SPINAL GANGLION.

Briefly, the spinal ganglion cells in the white rat form two groups distinguished, both by their chemical affinities and structural form, as has already been mentioned. The general form of the cell in section is somewhat oblong, this shape, however, is variable, and some of the cells are quite circular in outline. For the most part, the smaller cells which are surrounded by thick connective tissue or compressed among larger ganglion cell-bodies show triangular, polygonal or very rarely a spindle form. Very probably these irregular shapes result from the action of the preserving fluids.

The size of the cells in the cervical spinal ganglia (ganglia

[1] EWING, J.—Studies on Ganglion Cells. *Arch. of Neurol. and Psychopathology*, Vol. 1, No. 3, '98.

from cervical enlargement) is quite variable ranging from 55×46 μ to 19×17 μ in diameter. The following table shows the size of the cell-body and its nucleus respectively:

TABLE I—Showing the size of the spinal ganglion cells in the cervical ganglia of the adult white rat.

Cell-body	Nucleus	Cell-body	Nucleus
Series A			
51×50	19×15	56×40	18×15
60×48	15×15	58×45	18×16
56×47	17×15	59×50	18×15
55×35	19×15	55×50	16×16
50×50	20×15	50×45	19×15
Average size . .		55×46	18×15
Series a			
34×31	14×14	36×33	16×15
39×28	15×13	30×26	15×12
44×34	14×16	42×31	15×14
40×25	17×15	40×34	17×15
Average size . .		38×25	15×14
Series B			
3 ×24	13×11	25×25	14×13
26×25	12×12	25×20	12×11
25×24	13×12		
Average size . .		26×23	13×12
Series b			
19×15	10× 9	19×15	10×10
19×19	10×10	19×15	11× 9
20×19	11×10		
Average size . .		19×17	10×10

Series A.—Measurements of largest cells, two from each section, taken from five successive sections, 6 to 7 μ thick.

Series a.—Measurements of cells next in size to the largest. Two cells in each case from the same sections used for Series A.

Series B.—Measurements of cells next in size to Series a. One cell in each case from the same section used for Series A, a.

Series b.—Measurements of smallest cells. One cell in each case from its same section used for Series A, a, B.

There is also a third group of cells which we shall designate Series C. This contains two kinds of cells in size, one being nearly the size of those in Series a, and the other the

same size as those in Series B, but never being found as large as those in Series A or as small as those in Series b.

This material, taken from the white rat with a body weight of 140 grams was fixed with formalin-acetic-sublimate mixture (6) and stained by toluidin blue and erythrosin.

As is shown in Table I the size of the cell-bodies is variable; the smallest have nearly the same size as in a young rat just after birth, but the largest ones attain nearly three times their diameter. These measurements of spinal ganglion cells in the white rat correspond to those obtained by other investigators in this laboratory. Between these extremes, many cells intermediate in size are noticeable. Before going on to discuss the peculiarities of these cells, it may be well to compare the diameters of the spinal ganglion cells in other vertebrates with those in the white rat.

BUEHLER[1] measured the spinal ganglion cells in several classes of vertebrates. He obtained the following results:

TABLE II—Showing the diameter in μ of spinal ganglion cells in various vertebrates—after BUEHLER.

First column gives the average diameter.
Second " " " diameter of the largest cells.
Third " " " " " smallest cells.

Name	Average	Largest	Smallest
Fish (Leuciscus rutilis) 14 c m long	20	30	10
Frog	40–50	70–80	10–20
Lizard		25	20
Dove	30–40	50	12–20
Cat	50+	70–80	22
Rabbit	50+	70–80	16
Dog	50+	108	24
Man	45-67	120	20

He also noticed that the spinal ganglion contains a greater number of small than of large cells.

CAVAZANNI[2] also measured the diameters of spinal ganglion

[1] BUEHLER, A.—Untersuchungen über den Bau der Nervenzellen. *Würzburg*, '98.

[2] CAVAZANNI, E.—Sur les Ganglion Spinaux. *Arch. Ital de Biologie*, T. xxviii.

cells in several kinds of mammals in adult and embryonal stages, but does not give separate measurements for the small and large cells.

From the observation of these two authors, it is known that the spinal ganglia of all vertebrate animals thus far examined contain two kinds of the cells. The question arises, whether all of these cells are functional or not, and, if functional what is indicated by this variation in size? To assist in forming an opinion on this point let us first describe the internal structure of these cells and make a comparison between their structural differences.

III. INTERNAL STRUCTURE OF SPINAL GANGLION CELLS.

Different arrangements of the stainable substance in the spinal ganglion cells according to the different sizes of the cell-bodies have been noticed by several authors, and according to these arrangements, they have tried to classify the cells. Some of these classifications are as follows:

NISSL[1] found in spinal ganglion of rabbit, the different classes of cells which he has distinguished and recognizes the great differences in the size of the cell-body. He further notes a number of varieties of cells characterized by the size of the masses of stainable substance. Using this as a criterion he distinguishes (1) those in which the stainable substance consists entirely of large masses, (2) those in which it consists of very small masses, (3) those in which both large and small masses are present. In this case the two sizesof stainable masses can be present in equal numbers or either the smaller or the larger may be in excess. (4) Both large and small masses of stainable substance may be present with a peculiar arrangement of the masses of a given size thus imparting to the cell a characteristic appearance.

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[1] NISSL.—Ueber die sogenannten Granulæ der Nervenzellen. *Neurolog. Centralblatt*, '94, Nos. 19, 21, 22.

Ueber die Nomenclatur der Nervenzellenanatomie und ihre nächsten Ziehle. *Neurolog. Centralblatt*, '95, No. 23.

LUGARO[1] distinguishes in the dog five different varieties of the spinal ganglion cells.

I. Large clear cells with delicate, closely packed stainable masses which are distributed uniformly throughout the cell-body. Around the nucleus, the stainable masses are more closely packed. The nucleus is large, clear and is provided with a nucleolus. These cells appear to be numerous in spinal ganglion.

II. Clear, medium-sized cells with irregularly formed small and large stainable masses which are large at periphery. Even here we see that individual masses are not isolated but are united together by fine processes. The nucleus is clear and possesses a nucleolus. These cells are most numerous.

III. Small, dark cells with small numerous stainable masses. Larger masses lying in region of the nucleus. The ground substance becomes diffusely stained. The nucleus also stains diffusely and contains two or more nucleoli. These cells rank third in point of number.

IV. Small and medium sized clear cells with large stainable masses which are present in small numbers and connected with each other by processes. The nucleus freqently possesses more than one nucleolus. These cells are not numerous.

V. Large, clear cells with long drawn out masses which are continuous with one another and arrange themselves in concentric lines around the nucleus. These last cells present a laminated appearance like the cross section of an onion. These cells are least numerous.

LENHOSSEK[2] divided the spinal ganglion cells into the following three varieties:

The first variety consists of cells with a very pale ground substance only. These, which are the largest cells, have a dense ground substance and with less numerous and loosely arranged stainable masses, which are most dense around the nucleus.

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[1] LUGARO, E — Sulle alterazioni delle nervosi dei ganglia spinali. *Rev. di pathol. nerv. e ment.*, Firenze, Vol. V ('96) Nos. 8 and 12.

[2] LENHOSSEK—Ueber den Bau der Spinalganglienzellen des Menschen. *Arch. f. Psychiat. u. Nervenk.*, Berl., Bd. XXIX ('96-'97), S. 346-380.

To the second variety belongs a coarse granular cell (grōb-scholligen Zellen), the appearance of which depends on the appearance of the stainable substance, and most of the cells belong to this variety. These cells are of medium size, but sometimes small and rarely very large.

The third variety contains the spinal ganglion cells which have a peculiar internal structure. These cells stain darkly because of the density of the ground substance.

Cox[1] describes in the rabbit two main varieties of spinal ganglion cells:

One variety contains larger or smaller irregular masses of stainable substance, which do not show a distinct concentric arrangement. The cells of this variety may be either large or small.

The other variety contains large, irregular masses of stainable substance arranged concentrically.

From these citations it is clear that the above authors classified the spinal ganglion cells in accordance with the three following characters: (1) Size of the cell body; (2) The arrangement of stainable substance; (3) Chemical reactions of the ground substance. Using the characters just named we shall in this paper make still another classification which will be presented in detail below.

The smaller cells in the white rat measure only 25 to 18 μ in diameters, and the nucleus 12 to 10 μ in diameter. Such small cells are more numerous than the larger cells and stain more deeply. One group of these smaller cells stains so deeply with erythrosin and eosin that one can easily distinguish them from the larger ones. A careful study of these smaller cells which stain next deeply shows that the stainable substance is thickly massed around the peripheral portion of the cell. Near the nucleus, the stainable substance is very scanty. The stainable masses are of large size only at the extreme periphery of the cell and the remaining part is filled up with fine powdered

[1] Cox, W, H.—Der feinere Bau der Spinal Ganglienzelle des Kaninchens. *Anatomische Hefte,* Abth. 1, Bd. 10, '98.

granules. No "clear zone," which is present in the larger cells between the cytoplasm and the nucleus, is visible. For this reason the outline of the nucleus is less sharp and the cell-body appears somewhat structureless or homogeneous.

Besides these cells, still another variety of smaller cells is observable. The cell-body stains nearly as deeply as the small cells described in the previous paragraph. No clear zone is visible around the nucleus. The arrangement of the stainable substance resembles very much that of the larger cells. The masses of stainable substance are of large size and distributed through out the cell-body without showing any constant or fixed arrangement. Small masses of stainable substance are also noticeable throughout the cell-body. No such phenomenon as the accumulation of the stainable substance in large masses or the forming of the large sized stainable masses only at periphery is visible, as in the case of many other cells both large and small. The ground substance is dense. LENHOSSEK[1] called attention to this density of the ground substance in the following way:

"Die kleineren Zellen unterscheiden sich nun von den grösseren nicht nur durch ihre geringeren Dimensionen, sondern in sehr auffallender Weise auch durch ihre besonderes färberisches Verhalten. Im Allgemeinen kann man sagen, je kleiner eine Zelle ist, desto intensiver verbindet sie sich mit den meisten Farbstoffen, namentlich mit denjenigen, die das Protoplasma färben. Die Erscheinung ist hier nicht in der Gegenwart von besonderen Körnerbildungen oder drgl. begrundet, obgleich die kleineren Elemente in ihren Randschichten relativ gröbere Plasmaschollen beherbergen als die grossen, sondern hängt, wie ich mich diesmal mit Bestimmtheit überzeugt habe, in erster Reihe mit einer dichteren Beschaffenheit der Grundsubstanz des Protoplasma zusammen."

We can fully corroborate this observation of LENHOSSEK, but the writer noticed in many cases that the NISSL'S stainable

[1] LENHOSSEK—Centrosom und Sphäre in den Spinal Ganglienzellen des Frosches. *Arch. f. mikr. Anat. und Entwicklungsgeschichte.* Bd. XLVI, H. 2, '95.

substance in the powder form is also quite abundant in these cells. This contributes to the dark appearance of the cell-body. These cells just described are generally larger than those mentioned in the first paragraph. In the former case, the cell-body looks structureless. But the writer's observation shows, this is not homogeneous or structureless in the strict sense of the word as is indicated by the statement that this cells stain somewhat darkly because of density of the ground substance. A well preserved and satisfactorily stained preparation shows very clearly that the cytoplasm is filled up very densely with a peculiar substance named by HELD[1] the "neurosomes" which are stainable only by acid dyes. There are so many of these neurosomes that one can hardly distinguish one individual from another and the cytoplasm appears a continuous reddish mass. The small cells (in Series C) are in the same condition, having great numbers of neurosomes. From this is quite clear that the chromophilic cells, as named by NISSL, are peculiar in the arrangement of the neurosomes.

Another interesting point concerning the small cells is that in every case the cell-bodies skrink a little more than those of larger cells. My own mixture for preservation (6) gives the cells in a nearly normal size and no spaces appear around the cell-body in the case of the larger cells. But around the small cells such spaces are to be seen. This skrinkage has been interpreted as a pathological change but one can hardly believe that there are so many pathological cells in the normal spinal ganglion. It should be remembered that the spinal ganglion contains a greater number of the small cells than of those larger in size. We will explain later why the small cells tend to shrink.

Let us first compare these small and large cells as to the arrangement of the masses of stainable substance in them. These masses in the larger cells are arranged in a more complex

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[1] HELD, H.—Beiträge zur Structur der Nervenzellen und ihrer Fortsätze. Erste Abhandlung. *Arch. für Anatomie und Entwicklungsg.*, Anat. Abth. '95

way than in the smaller cells. The writer has distinguished three types of arrangement in the larger cells.

One type presents coarse and large masses which exist throughout the cell-body without showing any fixed arrangement. Clear spaces around the nucleus and at the extreme periphery of the cell-body are very distinctly visible (Fig. 1). Besides the coarse masses, minute powder-like granules are visible (see Fig. 2). This type of cell is very abundant.

A second type of these cells has large and coarse masses only at the periphery (Fig. 2), but the remaining part shows much smaller masses. The arrangemeut of masses of stainable substance is faintly concentric. The clear zones about the nucleus and at the periphery are visible. These cells are also very numerous in the spinal ganglion.

In the last type of cells, the stainable substance exhibits a beautiful concentric arrangement around the nucleus (Fig. 3). The stainable masses are rather large in size, although in some cases they may be small, and as in the first type the large stainable masses are distributed throughout the cell-body. The stainable masses show an elongated spindle form. As LUGARO'S picture shows, this type of cell suggests the cross section of an onion. When the level of section passes through the more peripheral portion of the cell-body, the stainable masses show a somewhat parallel arrangement, and sometimes they are continuous, making a strong heavy line. The clear zones are brilliant. This type of cells is not so abundant. The writer has classed these three types of the cells as larger cells (Table I, Series A and a).

Another type of cell was observed by the writer, the measurements for which are given in Table I, Series C. Curiously enough, this type of the cell-body stands between the larger and smaller cells as "intermediate" in structure. The structure of the cell-body is as follows: The masses of stainable substance distribute themselves throughout the cell-body in concentric or irregular layers. The size of the cell-bodies is variable; the larger cells measuring $38 \times 24\ \mu$ and the smaller $26 \times 21\ \mu$ in diameter. The most important and interesting

point is that the cell-body stains deeply with eosin or erythrosin as in the case of smaller cells. The clear zones are less differentiated than those of the larger cells. The cell-bodies do not skrink so much as those of smaller cells and sometimes do not show even a slight trace of shrinkage. For a better comparison we will summarize the various descriptions of the spinal ganglion cells which have just been given.

I. Larger Cells.

A. Table I, Series A and a, Fig. 1. The cells with large, coarse stainable masses which lie throughout the cell-body without showing a regular or constant arrangement.

B. Table I, Series A and a, Fig. 2. The cells with large, coarse stainable masses only at the periphery. Smaller masses fill up the remaining part.

C. Table I, Series A and a, Fig. 3. The cells with large, coarse stainable masses which lie throughout the cell-body, showing a regular, concentric arrangement.

II. Smaller Cells.

A. Table I, Series B and b, Fig. 4. The cell-body of small size, stainable masses accumulated at the periphery of the cell. The cell-body stains deeply in eosin and erythrosin. After fixing it has a tendency to shrink markedly.

B. Table I, Series B and b, Fig. 5. The cell-body is of small size. Stainable masses are large and distributed thoughout the cell-body. The cell-body stains very deeply as in the former case. There is no regular arrangement of the stainable substance. After fixing it has a tendency to shrink markedly.

III. Intermediate Cells.

A. Series C. The cell-body small or large size. The stainable masses are large and coarse and are distributed throughout the cell-body with or without showing a concentric arrangement. The cell-body stains deeply as in the case of smaller cells. These cells have a tendency to shrink slightly.

B. Series C. The cell-body is of small or large size. The

stainable masses are large at the periphery as in Fig. 2. The remaining part of the cell-body is filled up with small stainable masses. The arrangement of the masses is not regular. The cell-body stains deeply and has a tendency to shrink slightly.

This large number of varieties among the spinal ganglion cells calls for an explanation.

It seems to me very probable that the smaller cells, which were regarded as in a pathological condition or as artifacts (chromophilic cell) by some of the previous investigators[1] are in many cases the growing stages of the normal cells.

To support this view let us consider the evidences of growth in the spinal ganglion as determined by other investigators.

IV. REVIEW OF GROWTH CHANGES IN THE SPINAL GANGLION.

HODGE[1] counted in the frog the number of fibers in the posterior root and the number of the cells in the spinal ganglia of several nerves. From these observations he obtained the following results:

TABLE III—Showing the number of fibers and cells in the afferent spinal nerves of a Frog (probably Bull-frog). Weight not given but probably 150 grams in body-weight—(after HODGE).

No. fibers in dorsal root		No. of cells in ganglion.	Excess of cells	Ratio of one fiber to cells.
Seventh nerve (Right side)	1128	2767	1639	1:2.45
Eighth nerve (Left side)	1811	5416	3605	1:2.94
Seventh nerve (Left side) T's count	1364 1340	4456	3104	1:3.26

From the above table, we find that one afferent fiber of

[1] The word chromophile was first used by FLESCH to describe the cells which stain diffusely; later NISSL applied the term to the cells in which the stainable substance appears to be evenly diffused throughout the cell-body. This kind of the cells was considered by NISSL as pathological or an artifact (NISSL, *Allg. Zeschr. f. Psychiat.* etc., Berl. ('96), Bd. iii, S. 8).

[1] HODGE, C. F.—Some effects of electrically stimulating ganglion cells. *Amer. Journ. of Psychology*, Vol. II, '88.

the frog corresponds in these cases to from 2.45 to 3.26 of the spinal ganglion cells.

BUEHLER[1] obtained the following results from the examination of ninth spinal nerve of the frog (Rana esculenta). He found in dorsal root 680 fibers and in the spinal ganglion about 3500 cells giving a ratio of 1 to 5, while according to LEWIN[2] in the 32nd spinal nerve of the rabbit there were found only 3173 posterior root fibers to correspond to the 20361 cells of the spinal ganglion; a ratio of 1:6.4.

BIRGE[3] was able to show in the frog, first that the number of fibers found in the dorsal and the ventral spinal nerve roots increased as the frog increased in size, and second that in both the IInd and IXth nerves there was an excess of fibers in the trunk over and above the sum of the fibers in the two roots. This excess amounted to 16% in the case of the IInd nerve and 14%, for the IXth.

HARDESTY'S[4] studies on the spinal nerves of the frog gave us some important results. He ummarrizcd his observations as follows:

"1. The number of fibers in the ventral roots decreases from the spinal cord toward the spinal ganglion.

"2. The number of fibers in the dorsal roots decreases from the spinal ganglion toward the spinal cord.

"4. The section of the nerve trunk immediately distal to the spinal ganglion (dorsal branches excluded) contains a

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[1] BUEHLER, A.—Untersuchungen über den Bau der Nervenzellen. *Verhandlungen der Physik.-med. Gesellschaft zu Würzburg.* N. F. Bd. 31, p. 285, '98.

[2] LEWIN, VON TH.—Ueber die Zahlen der Nervenfasern und Ganglienzellen in den Spinal Ganglien des Kaninchens. *Centralbl. für Physiologie,* '96, Heft 15 und 16.

[3] BIRGE, E. A.—Die Zahl der Nervenfasern und der motorischen Ganglienzellen im Rückenmark des Frosches. *Archiv für Anatomie und Physiologie.* Physiologische Abtheilung. H. 5 und 6, 1882.

[4] HARDESTY, I.—The Number and Arrangement of the Fibers forming the Spinal Nerves of the Frog (Rana virescens). *J. of Comp. Neurol.,* Vol. IX, No. 2, '99.

greater number of fibers than are found in a section of the trunk further distal.

"5. The decrease in the number occurs among the smaller fibers of the nerve.

"6. The general explanation of these relations is found in the fact that the fibers arising from the spinal ganglion grow, on the one hand, toward the spinal cord by way of the dorsal root and, on the other hand, toward the periphery by way of the nerve trunk; and, that the fibers of the ventral root grow from the spinal cord towards the periphery.

"7. In frogs of increasing weight, the fibers of the dorsal root increase in number more rapidly than do those of the ventral root."

From the investigations of the above named authors it is evident that the number of cells in the spinal ganglia is greater than the number of fibers in the corresponding dorsal nerve root. This is true for the mammal represented by the rabbit, as well as for the frog. Further, HARDESTY has shown in the case of frog that fibers in the dorsal and ventral nerve roots, as well as in the nerve trunk, are distributed in different levels as though nerve fibers were continuously growing out from ventral horn cells on one hand and the spinal ganglion cells on the other, and he interprets his results as indicating growth. In mammals, however, DALE[1] was unable to find this indication of growth in the mature cat. If this arrangement in the cat should prove to be constant, the difference between the cat and the frog might be explained by long continued growth changes in the frog as compared with very rapid enlargement of the cat to a fixed size. Besides the excess of the cell-bodies in the spinal ganglion of both frog and rabbit and evidence of the growth changes in the frog, we have our own observation that the smallest and smaller ganglion cell-bodies are in the white rat most numerous and LENHOSSEK reports the same for the dog, and BUEHLER for the frog.

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[1] DALE—On some Numerical Comparisons of the Centripetal and Centrifugal Medullated Nerve Fibers arising in the Spinal Ganglia of the Mammal. *Journ. of Physiology* (FOSTER), Vol. XXV, No. 3, 1900.

HARDESTY has shown that in the frog it is the very small nerve fibers which represent those which are growing, while observations based on the GOLGI method show that in the spinal ganglia as a rule the size of the cell process is proportional to the size of cell-body, a larger cell-body sending off a larger process.

DR. DONALDSON[1] obtained the following results from his observations which were made upon growing nerve cells in the white rat, as they appear between birth and maturity. He says that "in the growing spinal ganglion of the lumbar nerves, the increase in volume of the largest ganglion cell-bodies was shown to be very closely correlated with the increase in the area of a cross section of the nerve fiber growing out of these cell-bodies." The following table shows the relations just mentioned more in detail:

TABLE IV—The relative volumes of the cell bodies and areas of the cross section of the nerve fibers of the growing white rat of different weights—After DONALDSON.

Body weight grams	Volumes of ganglion cells	Areas axis	Areas axis and sheath
4.7	1.0	1.0	1.0
10.4	1.6	1.4	2.8
25.7	4.9	4.6	9.3
68.5	11.2	12.2	24.0
159.0	15.0	14.4	29.7

From the above table, it is evident that the larger sized cell-body sends off larger sized processes, and smaller cells the smaller processes.

No author has ever counted the smaller and larger ganglion cells separately. HARDESTY[2] gave the table showing separately the number of small and large fibers forming the dorsal and ventral roots. This gives a general idea concerning the numerical relations between small and large cells in the frog.

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[1] DONALDSON, H. H.—The Functional Significance of the Size and Shape of the Neurone. *Journal of Mental and Nervous Disease*, Oct., 1900.

[2] HARDESTY, I.—Loc. cit.

TABLE V—Showing the small and large fibers separately in dorsal and ventral regions of spinal nerve of Frog (Rana virescens)—After HARDESTY.

In the following table, Sec. 1 and Sec. 3, mean the two different levels of sections. Small fibers are those 5 μ or less in diameter

Nerve		Fibers	Sec. 1	Sec. 3	Dif.
III N.	Dors	Small	155	164	9
		Large	162	165	3
	Vent.	Small	306	289	17
		Large	91	90	1
V N.	Dors.	Small	193	203	10
		Large	95	96	1
	Vent.	Small	45	39	6
		Large	88	88	0
VII N.	Vent.	Small	107	95	12
		Large	284	282	2

As the above table shows, the small fibers in some nerves are more numerous than the large fibers. It seems very probable that some of the small cells are still in an immature condition and have not yet sent off an axone, If this is true, then there must be a greater number of these small cells than is represented by small fibers in the nerve root.

From this numerical relation, it seems to me that small cells are in an immature or growing stage. This can be shown to be true in another way. The writer gave a hint in the preceeding pages that the small cells show a tendency to shrink readily. We know that a cell-body which contains much water is harder to preserve in a normal state than the cells with less water. The cells with more water show more shrinkage. It is already known that the animal body contains when young comparatively a greater quantity of water than the mature animals.

I am allowed to quote from some unpublished observations of DR. DONALDSON on the white rat at birth and at maturity which show the following percentages of water in the central nervous system.

TABLE VI—Percentage of water in white rat at different ages—After DONALDSON.

Age	Brain	Spinal Cord
Birth	87%	85%
Maturity	78%	72%

This decrease occurs gradually, and after the animal reaches its mature condition remains nearly constant. The high percentage of water in the whole nervous system indicates that the nerve cells probably contain much water; that is to say, it appears from this justifiable to conclude that nerve cells in the growing condition contain the larger quantity of water and since the cells with the larger quantity of water are most readily shrunken, the phenomena of shrinkage in the small cells does not signify a pathological condition, but shows that the cell body is physiologically immature, that is contains the higher percentage of water which is necessary for its growth.

Let us compare the structure of the small cells of the adult animal with the cells of a young rat. The difference in cell-size in the spinal ganglion appears during uterine life. In a white rat weighing 4.52 grams differences in size are already quite clear. The large cells attain the diameter of about 25 μ and the nucleus about 12 μ. On the other hand, the small cells only attain 14 μ in the diameter of the cell-body and their nucleus 8.5 μ. In this stage, the small cells have a comparatively large nucleus and little cytoplasm. The stainable substance is diffused through the cytoplasm in this early stage. In the white rat of 10.84 grams body-weight, the large cells attain 35 μ in the mean diameter and the nucleus 16 μ. The small cells in this stage measure 14.1 μ in the mean diameter of the cell-body and 9.7 μ in the mean diameter of the nucleus. In the rat weighing 10.84 grams the outlines of the large cells are sharply bounded and the cell-body stains strongly with methylene blue. The small cells, however, not only maintain nearly same size as at birth but also the cell-body shows the same appearance as in the case of the new-born rat.

In the next stage, namely in the rats of 25.1 grams in the body-weight, the large cells develop remarkably, attaining 39 μ in the mean diameter of the cell-body and 13 μ in the mean diameter of the nucleus. The small cells, on the other hand,

[1] The writer observed in the embryo of Amia calva in which difference already takes place when it is 6 mm. long.

develop hardly so fast, attaining 15 μ in the mean diameter of the cell-body and 10 μ in the mean diameter in the nucleus. In the later stages, as in the previous case, only the large cell grow rapidly while the small cells remain nearly the same size.

Finally, in the rats at maturity, the large cell-bodies attain 50 μ in the mean diameter while small cells are only 18 μ in the mean diameter.

On comparing the cell-size of the matured cells with those of animals just born, it appears that the large cells in the adult white rat attain about twice the diameter of those of a white rat just born. On the other hand, the small cells in the adult rat maintain nearly the same size as those in the new born rat, showing the difference of about 4 μ in mean diameter. These relations are presented in the following table:

TABLE VII—The large and small spinal ganglion cells in cervical ganglia of the white rat of different weights.

	Large		Small	
Weight	Cell-body	Nucleus	Cell-body	Nucleus
4.52	25 μ	12 μ	14 μ	8.5 μ
10.84	35	16	14.1	9.7
25.1	39	16	15	10.
68.8	37	17	14.4	10.
157.	50	17	18	10.

From the above observations, the following conclusion is reached:

(1) The differentiation in the size of the cell-bodies appears in the rat in early fœtal life.

(2) The diameter of small cells in an adult white rat is only slightly greater than that at birth.

(3) The internal structure of the small cells in an adult white rat shows the same appearance as that at birth.

(4) The nucleus remains relatively large: a striking character of immature nerve-cells.

BUEHLER[1] made the following suggestions concerning the

[1] BUEHLER, A.—Loc. cit., p.16.

small cells: "Es kommt, wie ich mich bei Frosch und Kröte und auch beim Kaninchen überzeugen konnte, physiologischer Weise zum Untergang speciell der grossen Spinalganglienzellen. Die Degeneration verlaüft in verschiedenen Formen und allem Anschein nach wenig rapid. Man sieht in einem Spinalganglion des Frosches ca. 20—25 untergehende Zellen, beim Kaninchen relativ noch viel weniger. Die verloren gegangenen Zellen müssen ersetzt werden, und dies geschieht Wahrscheinlich dadurch, das eine der Kleinen durch Wachstum ihre Stelle einnimt. Da nach dem frühesten Jungendstadium eine Vermehrung von Nervenzellen nicht mehr vorkommt, muss das Spinalganglion, um für die Zeit des Lebens functionsfähig bleiben zu können, in der Anlage genügendes Ersatzmaterial in Gestalt von Reservezellen mitbekommen. Genauere Untersuchungen hierüber zu machen, bin ich indess noch nicht in der Lage gewesen."

From this we see that BUEHLER considers that some of the largest cells degenerate during the life of the animal and that the place of the degenerating cells is taken by some of the small cells which have remained immature and ready to develop as the occasion demands.

LENHOSSEK[1] regards these small cells as immature or "Jugendlich." He most often found the centrosome in the cells of this type.

DOGIEL[2] studied the spinal ganglion cells with methylene blue which was injected into the living animal (dog, cat, rabbit and guinea pig). In these preparations he noticed two kinds of the spinal ganglion cells which he designated as I type and II type, respectively. To these types he gives the following characters:

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[1] LENHOSSEK—Centrosom und Sphäre in den Spinalganglienzellen des Frosches. *M. Schultze's Arch.*, XLVI, p. 345, '95.

[2] DOGIEL, A. S.—Der Bau der Spinalganglien bei den Säugethieren. *Anat. Anz.*, '96, Bd. XII.

DOGIEL—Zür Fräge über den feineren Bau der Spinalganglien und deren Zellen bei Säugethieren. *Internat. Monatschr. für Anat. und Physiologie*, Bd. XIV, '97, Heft, 4 und 5.

I type of Cells—This form of cell is represented by two varieties; one measures 77-175 μ[1] in its long diameter, while the breadth is 43-86 μ; the other measures 21-30 μ in its long diameter and the breadth is 12-25 μ. The latter appears by the method in smaller numbers. This variety of cell receives the axone from II type of cells. The fiber of this I type cell divides into two branches, forming the "T" shaped fiber. These cells are those ordinarily called the ganglion cells.

II type of Cells—The axone of such a cell breaks up within the ganglion into a large number of branches. The branches lose their myelin sheaths and terminate about the cells of I type, forming a pericellular basket. The second type receives the termination of axone from sympathetic ganglion cells. The cell-body measures 43-132 μ in long diameter, while its breadth is only 30-55 μ.

Slight differences occur, however, as regards the morphology of these two varieties under Type I. The fiber of the larger cells is sheathed by medullary substance or myelin in all cases, while the fiber of the smaller cells is destitute of myelin except in a few cases. When the fiber is destitute of the myelin sheath, the axone shows a varicose appearance. These observations by DOGIEL bear very directly on the suggestion of the writer that these small ganglion cells are not pathological, as has been maintained, but immature. For DOGIEL here shows (1) that some of the small cells give off axones of the typical spinal ganglion forms; (2) that the axone is often unmedullated, itself sign an immaturity, and (3) finally that the number of these cells from which no axone is to be traced is large and hence these are not to be considered as functional at this stage of development.

From these several observations, the writer concludes that the small cells of the spinal ganglion are in a growing state or in a more or less permanently immature condition. The grow-

[1] It is to be noted that the measurements of the spinal ganglion cells made by DOGIEL do not greatly exceed those given in Table II when the mean of the several diameters is taken.

ing fibers which are found in an adult frog might, therefore, very well be formed by the axones of these latent cell-bodies.

We have ventured to classify the spinal ganglion cells in a few groups but noticed that each group contains many varieties of the cells which were at first puzzling. These varieties appear to be the transitional stage.

If the spinal ganglion cells, are classified according to the measurements of size, then would follow:

I. Large cells (Series A and a); cells in completely mature stages.

II. Intermediate cells (equal in size to those in Series a and B); cells in both mature and immature stages.

III. Small cells (Series B and b); cells in immature stages.

VI. SUMMARY.

I. The spinal ganglion of the white rat contains two varieties of the cells; one variety is larger in size and stains lightly with eosin and erythrosin; another variety cells is smaller in size and stains deeply with eosin or erythrosin.

II. Besides the above two varieties, the spinal ganglion of the white rat contains one more distinct variety of the cells. This stands as "intermediate" in its structure and size between the two former varieties.

III. The small spinal ganglion cells which were described as chromophilic cells are considered by the writer as in an immature or growing condition, and not as pathological nor as artefacts as has been maintained by some of the previous authors.

IV. When the spinal ganglion cells of the white rat are classified according to size, into large, intermediate and small cells, these three groups are also found to be regularly graded in their internal structure.

DESCRIPTION OF THE FIGURES.

PLATE I.

The following figures were made by camera with same magnifying powers using the occular (No. 1) and the objective (1-12) of Bausch and Lomb.

Fig. 1. Large spinal ganglion cells from ganglia of the cervical enlargement of the white rat.

Fig. 2. Do Do

Fig. 3. Do Do

Fig. 4. Small spinal ganglion cells from ganglia of the cervical enlargement of the white rat.

Fig. 5. Do Do

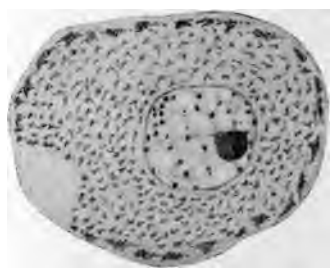

FIG. 2.

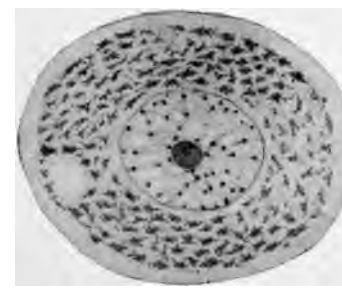

FIG. 1.

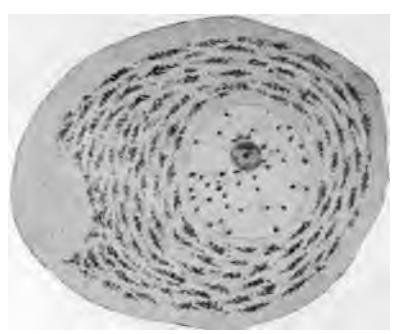

FIG. 3.

FIG. 4.

FIG. 5.

ON THE PRESENCE OF THE CENTROSOME IN CERTAIN NERVE CELLS OF THE WHITE RAT.

By SHINKISHI HATAI.

(From the Neurological Laboratory of the University of Chicago.)

With Plate II.

In the present paper, the writer describes the appearance of the centrosome in certain nerve cells of the white rat. The description is presented under the following heads:

I. Materials used and technique employed in this investigation.

II. The different groups of the cells in which the centrosome was discovered.

III. The morphology of the centrosome.

IV. Historical review of the centrosome in nerve cells.

V. Summary.

VI. Illustrations of the centrosome in the nerve cells of the white rat.

I. MATERIALS USED AND TECHNIQUE EMPLOYED IN THIS INVESTIGATION.

The materials used for the present investigation were taken from new-born white rats having a body-weight of 4.6-6.0 grams and adult white rats having a body-weight of 150 grams. Besides these animals, a young mouse (weight unknown) was used for comparison.

As a killing fluid, the writer's[1] new mixture (sublimate-acetic formalin mixture) was most frequently used. Besides this,

[1] HATAI, S.—Finer structure of the spinal ganglion cells in the white rat. *Journ. of Comp. Neurology*, Vol. XI, No. 1, 1901.

GILSON's acetic-nitric-alcohol mixture and CARNOY's acetic-chloroform-alcohol mixture were also used with advantage. VON LENHOSSÈK ('95) who first investigated the centrosome in the spinal ganglion cells of the frog used an aqueous solution of corrosive sublimate. DEHLER ('95) stated that osmic preparations give unsatisfactory results; nevertheless LEWIS ('96) has obtained very beautiful results from her osmic preparations of a certain annelid. HOLMGREN ('99) employed the fluid recommended by CARNOY. NELIS ('00), who studied mammalian nerve cells under pathological conditions for the purpose of demonstrating the centrosome, employed GILSON's fluid. The present writer obtained satisfactory results from all these fluids but most frequently employed his own mixture, not because it is necessarily superior to the others, but because the writer was most familiar with its action.

As staining agents, toluidin blue and thionin were used. The further procedure was the same as that given in the paper on the structure of the spinal ganglion cells.[1]

II. THE DIFFERENT CLASSES OF THE CELLS IN WHICH CENTROSOMES WERE DISCOVERED.

Before going on to describe the structure of the centrosome, the different classes of the nerve cells in which the present writer has found the centrosome may be here enumerated.

Two young white rats, having body weights of 4.6 and 6 grams respectively, and three adult white rats, each having a body weight of approximately 150 grams were used. The young rats were new-born. The regions examined from these animals were the cortex of the cerebrum, cerebellar cortex, corpus dentatum, motor cells in the ventral horn of the spinal cord and the spinal ganglia. The sections were cut 6μ in thickness. Three or four slides were made from each of the regions just mentioned. Every section on each slide was carefully examined. In this study it was observed that most of the nerve cells of young white rats possess a centrosome and a well

[1] loc. cit.

marked sphere. In the adult white rats, however, the nerve cells which contain these structures are by no means so abundant.

The following table shows the localities in which the centrosome was observed :

TABLE I. Showing the groups of the cells of the white rat in which centrosome has been found.

Age	Largest cells in cerebral cortex	Purkinje's cells	Nucleus of corpus dentatum	Motor cells in ventral horn spinal cord	Cells in spinal ganglion
Adult	present	present	o	o	present
Young	present	present	present	present	present

It is interesting to find that the centrosome is so widely distributed through the central nervous system, of the young as well as the adult white rat, while some authors—viz., NELIS, FLEMMING—claim that the centrosome can not be found in the normal nerve cells of the higher mammalia. In adult animals, the writer could not find the centrosome either in the nucleus of the corpus dentatum or in the cells in the ventral horn of spinal cord, although it was clearly to be demonstrated in these localities in the case of young animals. It is a reasonable supposition, however, that the invisibility of the centrosome in these two regions in the adult white rat is due to the large quantities of the stainable substance which fills up these nerve cells and thus obscures the presence of the centrosome. The writer noticed, very often, structures similar to the centrosome in both these regions; but was unable to determine its structure with certainty because not only the clear space but also the radial arrangements of cytoplasm, which is most characteristic for the centrosome, were obscured by the presence of many small granules of a uniform size.

The writer believes, however, that more improved methods both in preserving as well as staining would overcome these difficulties and reveal the centrosome in these localities also.

III. THE MORPHOLOGY OF THE CENTROSOME.

As is shown in the illustrations, the centrosome as well as the sphere[1] in PURKINJE cells and in the pyramidal cortical cells, lie, as a rule, at the base of a main dendrite near the nucleus (Figs. 2, 3, and 5-10). On the other hand, the centrosome and the sphere in the spinal ganglion cells lie, as a rule, on the side of the nucleus towards the axone hillock (Figs. 1, 11).

The centrosphere is somewhat circular in cross section with a less quantity of protoplasm. This gives the clear and transparent appearance to the centrosphere. A coarse granular layer of the protoplasm surrounds the centrosphere, which layer VON LENHOSSÈK termed the "plasmosphere." The stainable bodies (NISSL'S granules) are present in the plasmosphere, as in the remaining part of the cell-body, but not in in the centrosphere. These differences in the structure of the cytoplasm make it possible to distinguish the centrosphere from the remaining structures. The appearance of a radial arrangement of the microsomes originates from the centrosome which lies within the centrosphere (Figs. 3, 7, 8, 9, 10). These radial lines extend from the center into the plasmosphere. The lines are composed of a continuous series of minute protoplasmic corpuscles, smaller than the centrosome, which are known as microsomes (HANSTEIN, 1880). These radial lines formed by the microsomes are covered over very often, in the case of an adult animal, by the great number of the stainable masses which almost entirely obscure the radiations. We distinguish the microsomes from the stainable masses by the fact that the

[1] Different authors use different terms to designate the structure of the centrosome but the present writer employs the terms which were used by VON LENHOSSEK—centrosome, centrosphere and plasmosphere. The "centrosome" is a group of minute corpuscles which lie centrally in the centrosphere, or "centralscheibe." The "centrosphere" is the clear transparent area surrounding the centrosome. The "plasmosphere" is the area with coarse protoplasmic granules which surrounds the centrosphere. The plasmosphere grades into the surrounding portion of cell body and is not clearly separated from it. The present writer uses the term "sphere" to designate the entire structure, including centrosphere, radial arrangement of protoplasm and plasmosphere.

microsomes stain intensely with eosin or erythrosin, while the stainable masses do not take a color with those reagents but only with toluidin blue, thionin or methylen blue; that is to say, with the basic stains. The shape of the microsome is nearly circular in its outline, while the stainable masses are generally larger in size than the microsomes and very irregular in outline.

The diameter of the centrosphere is nearly constant measuring 3.4 μ to 4 μ and being approximately the same in the several groups of the nerve cells. The diameter of the centrosphere is not proportional to the diameter of the cell body in which it lies.

The writer noticed quite often a form of the centrosphere elongated in one axis and measuring 5 μ in a long diameter by 2μ in the short diameter. These are probably the regular centrospheres which have become distorted, for if one takes a mean diameter of the two measurements, then the diameter obtained is quite similar to that of the ordinary form, measuring 3.4 μ to 4 μ.

As has been mentioned already, the general appearance of the centrosphere is quite different from that of the rest of the cell body. This clearness in its appearance is greatly diminished by the growth changes in the cells as the animal matures. This fact will be discussed later on.

The position of the sphere in the cell is variable. Although, it lies, generally, near the nucleus (Figs. 1, 2, 3, 8, 9) in some cases, it may be somewhat removed from it (Figs. 5, 10, 11). The stainable masses are never visible within the centrosphere, and furthermore, the protoplasmic bodies within the centrophere are more minute than in the remaining part of the cell. These two facts in a large measure account for the clear and transparent appearance to the centrosphere. Besides this, in the protoplasm there are noticeable radial lines that, no doubt, originate from the centrosome which occupies the central position in the centrosphere. The number of the radial lines varies in the different cells.

The minute corpuscles or the centrosome bodies, larger than the rest of the microsomes, are in every case located centrally in the centrosphere. The corpuscle is a somewhat roundish body which stains very deeply with eosin or erythrosin as in the case of the microsomes. The number of the corpuscles which form the centrosome is variable, ranging from one or two (Figs. 1, 2, 3, 5, 7, 8, 9, 10) to a greater number (Fig. 4, 6, 11). Two corpuscles, however, occur in most cases. When the corpuscles occur in great number, they lie close together filling up the central area within a certain limit. It is very doubtful whether we should regard these numerous corpuscles as equivalent to the simpler centrosome composed of one or two corpuscles, because in these cases, the size of the all minute corpuscles is not similar and furthermore, all the corpuscles do not stain with the same intensity, one or two corpuscles which are located most centrally in these groups being distinguishable from the remainder. For this reason, the present writer suggests that some of the corpuscles are nothing more than ordinary microsomes which swell abnormally and are also located accidentally in the neighborhood of the centrosome. This interesting fact will be discussed later. Very often, the writer observed only one corpuscle within the centrosphere (Fig. 7). This appearance has been described by almost all investigators who have studied the centrosome in the nerve cells but this may be due to the fact that the plane of the section passes between the two corpuscles.

Fig. 1 is a section through a spinal ganglion cell of an adult white rat weighing 150 grams. The material was preserved with GILSON'S fluid followed by iron hæmatoxylin and bordeaux red. In this case, the sphere lies very close to the nucleus as BÜHLER figured it in a spinal ganglion cell of a frog. The radial arrangements of the protoplasm exist only at the region of the plasmosphere but not within the centrosphere as is the case in the embryonic cell. The centrosphere is somewhat oblong in shape with two conspicuous centrosomes in the central region. This area, "the centrosphere," is clear in its appearance with minute microsomes sometimes densely and

sometimes sparingly arranged but never regularly placed. The above structure of the sphere coincides with that found in other cell groups of the adult rat.

Fig. 2 was drawn from a large pyramidal cell in the cerebral cortex of the adult white rat. Fig. 5 is from a PURKINJE cell of the adult white rat. It is clear from these figures of the two different cells that the structure of the sphere in them is similar to that in the adult spinal ganglion cell.

Fig. 3 represents a large pyramidal cell in a young white rat. This specimen was preserved with the writer's new mixture followed by toluidin blue and eosin. If we compare this figure (3) with the figure 2. which was taken from the same locality of an adult white rat, the structural difference of the centrosphere is plainly seen. In the former case (Fig. 3) the radial arrangement of the microsomes is very conspicuous and unlike the latter case (Fig. 2) in which the internal structure presents a more homogeneous appearance.

Curiously enough, when the centrosome consists of a greater number of corpuscles, then the radial structure of the microsomes within the centrosphere is not clear but rather presents the homogeneous appearance as in the case of the centrosphere of an adult animal. This condition is shown in Figs. 4, 6, 11. The relation existing between the radial structure of the microsomes and the central corpuscles requires further study.

IV. HISTORICAL REVIEW OF THE CENTROSOMES IN NERVE CELLS.

The presence of the centrosomes in nerve cells was first announced by VON LENHOSSÈK[1] in the spinal ganglion cells of the frog. It had been previously noticed by several investigators that many spinal ganglion cells, especially the cells in which the nucleus occupied an eccentric position, showed a somewhat concentric arrangement of the protoplasm on the side toward the axone hillock. VON LENHOSSÈK noticed the minute corpuscles which are located in the center of this protoplasmic area

[1] VON LENHOSSEK.—Centrosome und Sphäre in den Spinal Ganglienzellen des Frosches. *Arch. für Mikr. Anat.*, Band XLVI, pp. 345-368.

and also showed the peculiar characters both of color and of size exhibited by these microsomes. He identified these corpuscles as the centrosome: "Das central Körnchenhauflein, ist ohne Frage identisch mit den Centralkörperchen VAN BENEDEN'S." The protoplasmic area which shows a concentric arrangement was called by this same investigator the "plasmosphere." He further distinguished within the plasmosphere, the definite area with a less quantity of the protoplasm and with somewhat transparent appearance, which he termed the "centrosphere." But he does not mention a radial arrangement of the protoplasm.

Soon after VON LENHOSSÈK'S discovery of the centrosome, many investigators reported their observations on the centrosome in many different classes of animals. The centrosome was found by DEHLER[1] ('95) in the sympathetic ganglion cells of frog; by BÜHLER[2] ('95, '98) in the spinal ganglion cells of the frog ('98) and in the nerve cells in the brain of the lizard ('95); by LEWIS[3] ('96, '98) in the giant nerve cells of an annelid which belongs to the Malduiae; by MCCLURE[4] ('96) in the ganglion cells of Helix pomatia; by SCHÄFFER[5] ('96) in the ganglion

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[1] DEHLER, A.—Beiträge zur Kenntniss von feineren Bau der sympathischen Ganglienzellen des Frosches. *Arch. für Mikr. Anat.*, Bd, xvi ('95), p. 724-729.

[2] BUEHLER, A.—Protoplasma-structur in Vorderhirn Zellen der Eidechse. *Würzburg*, '95.

BUEHLER, A.—Untersuchungen über den Bau der Nervenzellen. *Königl. Hof.-und Univ. Verlag.*, '98.

[3] LEWIS, M.—Centrosome and sphere in certain of the nerve cells of an Invertebrate. *Anat. Anz.*, Bd. xii ('96), S. 291-299.

LEWIS, M.—Studies on the central and peripheral nervous systems of two polychæte annelids. *Proceed. of the American Acad. of Arts and Sciences.* Vol. xxxviii ('98), No. 14.

[4] MCCLURE, C. F. W.—On the presence of centrosome and attraction spheres in the ganglion cells of Helix pomatia, with remarks upon the structure of the cell-body. *Princeton College Bulletin*, Vol. viii ('96), No. 2, p. 38-41.

[5] SCHAEFFER, I.—Ueber einen neuen Befund von Centrosomen in Ganglien und Knorpelzellen. *Sitzb. d. Acad. d. wiss. Wien.*, Math-nat., Cl. '96.

cells of Petromyzon; by AYERS[1] ('96) in the motor cells of the electric lobes of Torpedo; by VON KÖLLIKER[2] ('97) in adult human giant pyramidal cells; by HAMAKER[3] ('98) in the nervous system Nereis virens, SARS; by HOLMGREN[4] ('99) in the spinal ganglion cells of Lophius piscatorius; by NELIS[5] ('00) in the spinal ganglion cells of cat, dog, and pig under pathological conditions.

We may add a few words concerning the position of the centrosome. As was mentioned already, VON LENHOSSÈK discovered the centrosome in the concentric protoplasmic area, which is visible in the spinal ganglion in which the nucleus lies eccentrically. These peculiar concentric areas lie on the side of the nucleus toward the axone hillock and at a distance from it. DEHLER has found the centrosome in the same locality as VON LENHOSSÈK, although he has noticed a smaller number of central corpuscles than the former investigator. Recently BÜHLER and HOLMGREN have expressed a view opposed to that of VON LENHOSSÈK, maintaining that the concentric area of the spinal ganglion cells is a locality where the fibrillar structure of the protoplasm which is continuous with the axis cylinder runs from the axone hillock toward the nucleus forming an area in which there appears a somewhat concentric arrangement. They did not find the centrosome in that area but in the immediate neighborhood of the nucleus. According to these investigators, the centrosome as well as sphere of VON LENHOSSÈK are nothing

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[1] AYERS, HOWARD—The origin and growth of brain cells in the adult body. *Jour. of Comp. Neurology*, Vol. vi ('96), No. 3.

[2] VON KÖLLIKER, A.—Handbuch der Gewebelehre des Menschen. Bd. ii ('97), *Leipzig*, p. 812.

[3] HAMAKER, J. A.—The nervous system of Nereis virens, Sars. A study in comparative neurology. *Bull. of the Mus. of Comp. Zool. at Harvard Coll.*, Vol. xxxii ('98), No. 6, pp. 89-124.

[4] HOLMGREN, E.—Zur Kenntniss der Spinalganglienzellen von Lophius piscatorius. *Anatomische Hefte*, Bd. xii, Heft 1, '99.

[5] NELIS, C.—L'apparition des centrosome dans les cellules nerveuses au cours de l'infection rabique. *Le Névraxe*, 1900, Vol. 1, Fasc. 1.

more than the ordinary microsomes which stainedaccidentally so as to appear similar in color as well as form to the centrosome. The present writer has found the centrosome, in most cases, just near the nucleus as it was described by BÜHLER, HOLMGREN, etc. The eccentric position of the nucleus was noticed very often by the author. The centrosome, however, occurs not only in such a cell but also the cells with centrally located nuclei. From this observation, we can say that the position of the nucleus is not of primary importance for the occurrence of the centrosome.

The number of the central corpuscles or centrosomes in the strict sense of the word has been given differently by different investigators. VON LENHOSSÈK has counted numerous corpuscles within the centrosphere to which collectively he gave the name of centrosome. DEHLER and MISS LEWIS have also noticed several corpuscles, although they counted only one or two in some cases. BÜHLER is the first investigator to give the number of the corpuscles forming the true centrosome as two. NELIS[1] observed that the centrosome is composed of only one corpuscle in a resting state. When the cells are preparing to divide, however, under certain conditions, the centrosome may also divide into two. Very often he has found the two centrosomes in the cells one on each side of the nucleus. The present writer noticed that the centrosome consists of two minute corpuscles in most cases, but many corpuscles occur occasionally, and in one instance there was only one. In the preceding chapter it was stated that when the centrosome is formed of many corpuscles the structure of the centrosphere is modified in such a manner that the protoplasmic relations in the centrosphere become invisible and it presents a homogeneous appearance as shown in the adult nerve cells, the centrosphere being filled with microsomes. In this case, the radiation in the plasmosphere is either destroyed or it still retains its characteristic arrangement as far as these preparations are concerned.

[1] NELIS stated that the centrosome is wholly invisible in the resting state of nerve cells, but under centain stimulation it may appear, in which event it is composed of one corpuscle.

VON KÖLLIKER is the first to report the centrosome in the mammalian central nervous system. He found the centrosome in a giant pyramidal cell of the posterior central gyrus of a man thirty years old. Later, NELIS demonstrated the presence of the centrosome in the nerve cells of some adult higher mammalia, but only under pathological conditions. He summarizes his observations as follows: "A l'état normal, un centrosome n'est pas visible dans les cellules nerveuses des mammifères. Le processus pathologique rabique détermine l'apparition du centrosome dans les cellules nerveuses des ganglions spinaux chez le chien et chez le lapin. Au cours de cette infection, le centrosome ne reste pas inerte; il semble se diviser; en deux; les deux centrosomes tendent à se sepàrer et à emigrer dans deux directions opposèes.

"Les modifications nucléaires des cellules nerveuses au cours de l'infection rabique représentent pour nous des phénomenès de règression, d'atrophie, prècèdés d'une tendance à la proliférations qui averte prèmaturèment, tendance se traduisant uniquement par l'apparition du centrosome au sein du protoplasme, sa division probable et le commencement de migration des deux centrosomes nouvellement formés."

He absolutely denied the presence of the centrosome in mammalian nerve cells in a normal condition as far as his observations went. He further mentioned a possibility of the division of nerve cells in the mature animals, although he has not actually observed it. On the other hand, MISS LEWIS' observations led her to doubt the dividing process in the adult nerve cells. But she does not base her statement upon the structure of the centrosome but on the fact that she has not noticed such cases of division although she has examined the preparations made from more than a dozen worms. The present writer also doubts the occurrence of division (mitotic) of matured normal cells in the normal condition for the following reasons: The sphere in an adult nerve cell is slightly different from that of young nerve cells. The centrosome in young cells shows a clear outline with a radial arrangement of the protoplasm, the radii originating from the central corpuscles or centrosome. The

clear space, or centrosphere, which surrounds the centrosome shows a definite figure in young cells. The structures above mentioned, however, are not to be seen when the cell attains a mature stage, and the radial arrangement of the protoplasm within the centrosphere not only becomes invisible, but the clear area, or centrosphere itself, also is dimished more or less in its diameter. In some cases, the radial arrangement of the protoplasm in the region of the plasmosphere is entirely absent (Fig. 11). These structural differences between an adult and young stage signify, no doubt, the slow atrophy of the sphere. If my statement is true, we can hardly imagine that the centrosome which is in process of degeneration may undergo division in a normal condition. The writer has examined a large number of the nerve cells of the white rat in which the centrosome is nicely demonstrable but has not noticed even a slight trace of the process of division.

These observations raise the whole question of the division of mature nerve cells.

The current theory claims that after a nerve cell once sends out its axis cylinder it is incapable of division or further reproduction. It is known, however, in the case of another kind of tissues, that under a certain pathological condition the matured cells produce daughter cells by the dividing process, either mitotic or amitotic. This phenomenon has been reported in the nerve cells also, but in our opinion the results are not fully established. TEDESCHI[1] who studied the results of intoxication on the mammalian body has observed the presence of karyokinetic division in the adult nerve cells. According to him this phenomenon occurs three days after the poisonous substance has been introduced into the body. Later NELIS[1] suggested the possibility of the division of an adult nerve cells under the

[1] TEDESCHI, A.—Anatomische-pathologische und experimentelle Untersuchungen über die Regeneration des Nerven Gewebes. *Vergl. Mith. Centralbl. f. allg. path. u. path. Anat.*, Jena, Bd. viii ('96), p. 449-451. Also Anatomische experimentellen Beiträge zum Studien der Regeneration des Gewebe des Centralnervensystem. *Beitr. z. path. anat. u. z. all. path.*, Jena, '97, Vol. xxi, p. 43-72.

[1] NELIS, C.—Loc. cit.

pathological condition. He said "Au cours cette infection, le centrosome ne reste pas inerte, il semble se diviser en deux, les deux centrosomes tendent à se sepàrer et à emigrer dans deux directions opposèes."

Recently some very interesting papers have appeared concerning the reproduction of the nerve cells. AYERS,[1] who is one of the authors, has summarized the observation made on the electric lobes of Torpedo, in his preliminary report, as follows:

"(1). Large motor cells (electric lobes), not to be distinguished from the ordinary functional cells except by the size of the nucleus and cell-body.

"(2). Cells of the same size as (1) but with two nuclear bodies. Both may be close together in the center of the cell or widely separated and lying near the periphery of the cell.

"(3). Cells showing an evident constriction of the protoplasmic body between the nuclei as though about to divide.

"(4). Double cells with short connecting bar which are usually large and band-shaped.

"(5). Double cells in which the connecting bar is drawn out into a thin filament, tapering conically from either cell-body towards the other.

"(6). Since each nerve cell of the brain and ganglia has a perilymphatic capsule surrounding it, when the cell-body is cut into two the perilymphatic space is not at once doubled but the two cells still lie in a common cavity. Because of this it is possible to trace the genetic relation of these electromotor cells even after they have completely separated by the breaking of the connecting bands, as in those cases where the nerve cells become completely separated. Ultimately, of course, the lymphatic spaces divide also by completing the capsular wall close about each cell."

The writer has noticed the same appearance as those described by DR. AYERS. Here, however, arises the very difficult question of interpretation, whether these cells which are

[1] AYERS, H.—Loc. cit.

apparently dividing are remnants of imperfect division of embryonal germ cells which have become functional in such a condition without any further morphological changes, or whether, on the contrary, they are formed by the division, either mitotic or amitotic, of functional adult cells under special conditions. If the foregoing supposition is true, then the phenomenon of the continuity of the nerve cell bodies does not prove the division of the nerve cells in a matured condition. Since at the present moment there is not the slightest direct evidence for the amitotic division of nerve cells, we are justified in demanding that good evidence of mitosis as shown by the condition of the nuclear substance, be brought forward—if these cells are to be considered as actually dividing. This has not been done.

In order to give a positive answer to the questions above mentioned, it seems to me that the only safe and reliable method consists in counting the nerve cells in the spinal ganglia of a given species of animal at different ages and thus determining whether there is any increase in their number. The present writer is trying this method under the direction of PROF. DONALDSON, and hopes to report in the near future. We can only say, at present, concerning the division-problem that the nerve cells in vertebrates as well as invertebrates have the centrosome and the sphere, which are regarded as the dynamic centers of the mitotic division and further that this centrosome is able to take the first steps of division under a certain forms of stimulation, as has been observed by some investigators; but in the normal state the centrosome in an adult nerve cell presents slight morphological differences from that of the embryonic cell, which we interpret as the beginning of degeneration.

V. SUMMARY.

(1). Several classes of nerve cells in the young rat, as well as adult rat, have a centrosome and attraction sphere.

(2). The centrosome in the young rat is more easily distinguished from the rest of the structures than that in the adult.

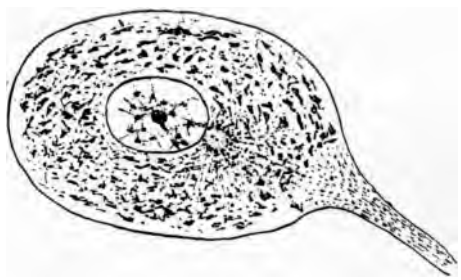
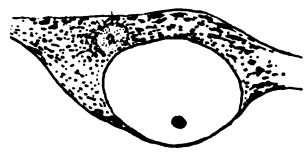


Fig. 1.



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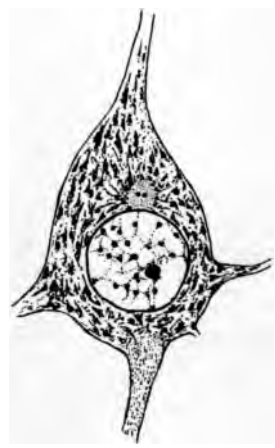

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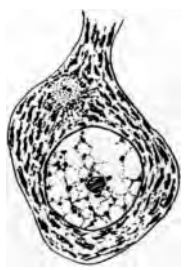

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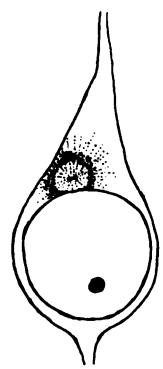
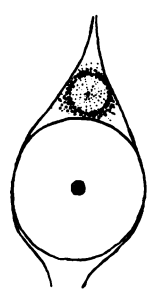


Fig. 3.

Fig. 8.



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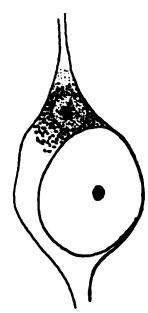

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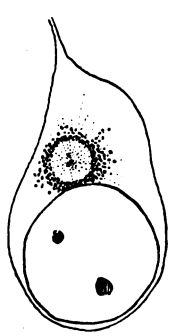

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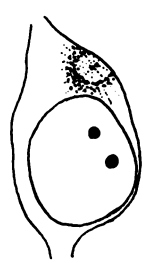

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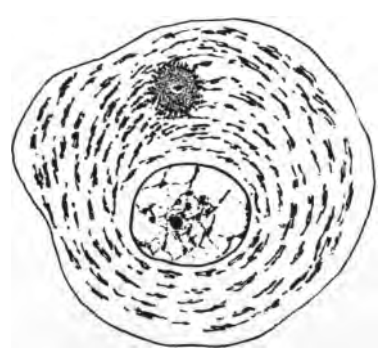

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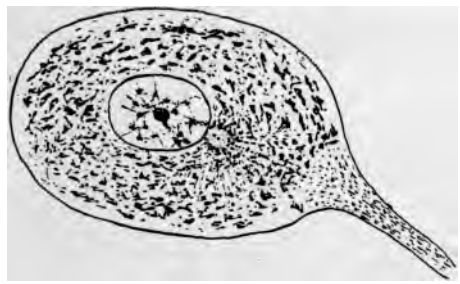
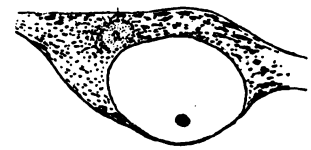


Fig. 1.



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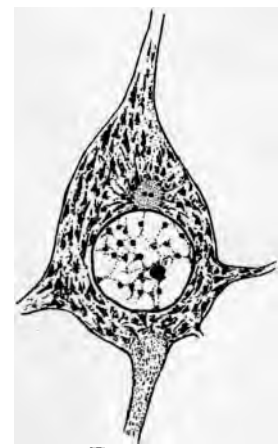

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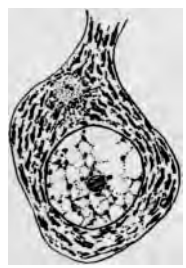

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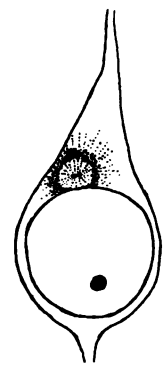

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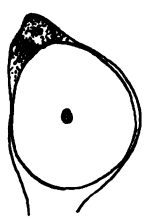

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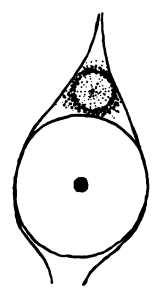

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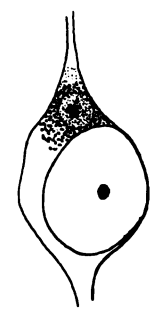

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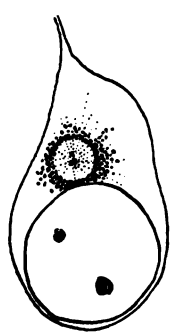

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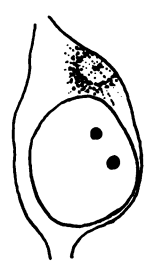

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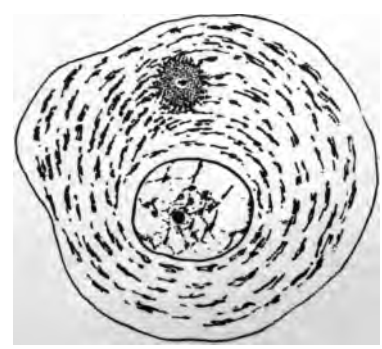

Fig. 11.

ON THE MITOSIS IN THE NERVE CELLS OF THE CEREBELLAR CORTEX OF FOETAL CATS.[1]

By SHINKISHI HATAI.

(*From the Neurological Laboratory of the University of Chicago.*)[1]

With Plate XVIII.

I. Introduction.

In the immature and growing mammalian nervous system, mitoses are very widely distributed. Mitotic changes in the cells of the central nervous system also occur in mature mammals under pathological conditions resulting from intoxication and mechanical lesions. FRIEDMAN,[2] MARINESCO[3] and TEDES-

[1] This work was begun at the Biological Laboratory of the University of Cincinnati and finished in the Neurological Laboratory of the University of Chicago.

[2] FRIEDMAN: *Arch. f. Psychiatrie*, Bd. XIX, '88; Bd. XXI, '89.

[3] MARINESCO: *Semaine Méd.*, '94, n. 29.

CHI[1] have reported the former, and GOLGI[2] and LEVI[3] the latter. To determine what the value of the mitotic changes were when pathologically induced, it was desirable to gather more details as to the changes in the nucleus during normal mitosis, and to this end the present investigation was undertaken.

II. Materials used and technique employed for the present investigation.

For the present investigation, foetal cats were exclusively used, the foetuses having body length (from the tip of the head to the origin of the tail) of 6.3 cm. At this stage of development they present abundant mitotic figures in almost every part of the encephalon, especially in the cerebellar cortex and the lining layer of the lateral ventricles of the hemispheres. Single sections of spinal cord of these cats showed rarely more than one or two dividing cells, and sometimes none were present. Five foetuses of the same litter were used for this investigation. As a preserving agent, GILSON'S fluid, CARNOY'S mixture, and RIPART and PETIT'S fluid were employed in each case. The following is a formula recommended by RIPART and PETIT:[4]

Camphor water (dilute) . .	75 grams.
Distilled water . . .	75 grams.
Crystalized acetic acid . .	1 gram.
Acetate of Copper . . .	0.30 gram.
Chloride of Copper . . .	0.39 gram.

(Osmic acid may be added to the fluid of RIPART and PETIT to increase the fixing power.)

In each case the fluid was used according to the direction

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[1] TEDESCHI: Anatomisch-pathologische und experimentelle Untersuchungen über die Regeneration des Nervengewebes. Vor. Mitth. *Centralbl. f. Allg. Path. u. path. Anat.*, Bd. VIII ('96); also Anatomische-experimentelle Beiträge zum Studien der Regeneration des Gewebes des Centralnervensystems. *Beitr. z. path. Anat. u. z. Allg. Path.* '97, XXI.

[2] GOLGI: *Berliner Klin. Wochenschr.* '94, p. 325.

[3] LEVI: Ricerche sulla capacità proliferativa della cellula nervosa. *Rev. di patol. Nerv. e Ment.*, '96, f, 10; also Sulla cariocinesi delle cellule nervose. *Rev. di patol. nerv. e ment.*, '98, f. 3.

[4] This formula is given in LEE'S Microtomist's Vade Mecum.

given by the authors. GILSON'S fluid always gave satisfactory results.

The materials preserved with the fluids mentioned, were carried through graded alcohols and embedded in paraffin in the usual way. The sections were cut three micra in thickness.

As the staining reagents, HERMANN'S saffranin and gentian violet, BIONDI-EHRLICH'S tri-color mixture, toluidin blue in aqueous saturated solution followed by erythrosin of 1% solution in 70% alcohol, and HEIDENHAIN'S iron-haematoxylin followed by either Bordeaux red or orange G in 1% solution in distilled water, were employed in each case.

In general, HEIDENHAIN'S iron-haematoxylin followed by Bordeaux red or orange G gives a very satisfactory staining of the mitotic figures. However, for the structure of the chromatic thread, for the relations of linin to the chromatin, and especially for the peculiarities of the nucleolus in active division, as well as during the resting stage, HERMANN'S or BIONDI-EHRLICH'S staining method gave more satisfactory results. The writer used also NISSL'S methylen-blue method, but the results were less satisfactory than those obtained from the fluids just mentioned. In each case, the sections should be stained slowly by long immersion in dilute solutions; otherwise, artifacts are obtained.

III. Germinal cells, or "Keimzellen."

In an early stage of development, the central nervous system is formed by a long continuous tube composed of several layers of the epithelial cells derived from the ectoderm. The wall of the neural tube soon becomes thicker as a result of the proliferation of the ectodermal cells of the primitive tube. This prolifieration is due to the division of the cells which lie near the lumen of the tube. These cells were called germinal cells or "Keimzellen" by HIS.[1]

Whether at some time in their history all the ectodermal cells which form the primitive neural tube un-

[1] HIS: Die Neuroblasten und ihre Entstehung im embryonalen Mark. *Abhandl. d. Nat. Phys. Kl. d. Königl. Sächs. Gesellsch. d. Wiss.* Bd. 15, '89.

dergo mitotic division, or whether some of the cells only have this power, is not definitely known. If the latter supposition is true, then the real relations of these cells to the remaining part of the ectodermal cells must be one of the important subjects to be investigated. It has been claimed by HIS that the germinal cells are fundamentally different from the rest of the epithelial cells. This idea of HIS, however, has been opposed by many investigators (VON KÖLLIKER,[1] VIGNAL, SCHAPER,[2] etc.)

SCHAPER has reached the same conclusion as VON KÖLLIKER, urging that the germinal cells are really young proliferating forms of epithelial cells which first gave rise to indifferent cells. These latter may be further differentiated, either into nerve cells or into glia cells.

On the assumption that the germinal cells produce two kinds of tissues, either nerve cells or glia cells, the primitive form of these two kinds of cells has been named by HIS neuroblasts and spongioblasts respectively. The early differentiation of these cells will not be discussed by this paper. The observations here recorded apply exclusively to well characterized germinal cells.

The germinal cells of the cerebellar cortex of foetal cats, present a very characteristic appearance with their circular outline, and well stained chromosomes. The size of the cell-body is variable according to the phase of nuclear change which is exhibited. The measurements of the cell-bodies will be given in the descriptions of the several phases.

The various phases of the germinal cells in their development may be described advantageously in the following order:

A. Germinal Cell in the Prophase. In the earliest stage of this phase (Fig. 1), the germinal cells assume a nearly spherical shape. The cell-body[3] enlarges slightly, measuring from

[1] VON KÖLLIKER: Handbuch der Gewebelehre des Menschen, Bd. II, '96.

[2] SCHAPER, A.: Die frühesten Differenzirungsvorgänge im Centralnervensystem; Kritische Studie und Versuch einer Geschichte der Entwicklung nervösen Substanz. *Arch. f. Entwicklungsmechn. d. Organ.* Bd. V, '97.

[3] There occurs considerable variation of the size of the cell-body as well as nuclear area, in each phase. The present investigation applies exclusively to the cells of large size.

4 × 3.6 micra to 6.3 × 6.3 micra in diameter. Moreover, germinal cells contain chromosomes more deeply stained than those of the resting epithelial cells, presenting bluish red tints after HERMANN'S method.

The cell-body, as well as the nuclear membrane, is distinctly visible in the germinal cells. The chromosomes occur in great abundance and are distributed throughout the nucleus. The linin is clearly distinguishable from the rest of the other structures, while in the epithelial cell, it is extremely hard to distinguish it. The most conspicuous, as well as most important changes in this phase, are the nucleolar movements. In the inactive epithelial cell, the nucleolus appears as a compact and homogeneous structure, lying within the nucleus (Fig. 24). This simple structure, however, separates into numerous granules, owing to a solution of the linin which intimately surrounds the nucleolus in its resting stage (Fig. 24). The structural relations of these small granules in the nucleolus will be discussed later.

Following this stage just mentioned, a most remarkable change is exhibited by the chromosomes.[1]

As figure 2 shows, the chromatic granules enlarge considerably, and at the same time a diminution in the number of the granules takes place. The chromosomes in this stage stain more intensely than in the first case, and also the reddish color predominates over the bluish (HERMANN'S method). The cell-membrane, the nuclear membrane, and the nucleolus, are clearly visible, although in some cases, the nucleolus dissolves into numerous minute corpuscles. The linin network, in which the chromatin granules are suspended in the resting stage, becomes distinct. The centrosome lies at one side close to the nucleus.

Fig. 3 shows some very important changes of the chromatic granules. The chromatic granules, which are scattered throughout the nucleus in the former stages, now arrange them-

[1] In this stage the cell-body, measures about 9 × 6.3 micra to 12.6 × 7 in diameter, while nucleus measures 5.4 × 5.7 micra to 11.7 × 7 respectively.

selves in a regular manner so as to make a continuous winding thread; this is the "spireme stage." This thread which carries the chromatic granules does not maintain the same diameter along its entire course, but is divided into numerous internodes by the larger granules which are suspended at regular intervals along the thread. The number of the small chromatic granules lying in the internodes, as well as the length of each internode, is variable. The whole number of internodes, however, seems to be always constant. In many instances, in this stage, the nucleolus, as seen in the resting state, does not appear. The cytoplasmic part of the cells stains very faintly, and is hardly visible. The thread which carries the chromatic granules is formed probably by two different substances. One of these is the linin which directly surrounds the granules, although it stains very faintly, and another substance which intimately surrounds or ensheaths the entire structure (linin and chromatic granules combined). Both of these are derived from the nucleolus. Very careful observation shows that the real chromatin substance stains an intense bluish red color, while the rest of the thread takes a bright red color after HERMANN'S method. The nucleolar substance not only covers over the spireme, but also is scattered throughout the nucleus, forming a network (Fig. 3). The centrosome lies at one pole of the oval nucleus. The spireme later accumulates at the center of the cell-body, as Figure 4 shows. In this stage, the most important change of of the mitotic figure is that shown by the chromatic granules. As is shown in Fig. 3 and also is still partially visible in Fig. 4, the chromatic granules aggregate, forming larger masses of the same substance. Whether each large mass of the chromatin corresponds to each of the internodes of the thread respectively, is not clear. These chromatic masses are somewhat bean-shaped and stain an intense bluish red color. The nucleolar, as well as linin substance which forms the thread in an early spireme stage ensheaths its chromatin, as Fig. 4 shows. The gradation from spireme to that of the aggregated form of the chromatin (Fig. 6) is beautifully shown in this figure. Another important

change occurring in this stage is a disappearance of the nuclear membrane.

Fig. 5 shows the completion of the former stage, presenting sixteen large masses, one of which exhibits a dumb-bell shape. The large chromosomes thus formed by the accumulation of the smaller chromatic granules are disconnected from one another. These separate bean-shaped masses next become somewhat dumb-bell shaped; then these masses fuse together end to end, thus forming again a long continuous strand (Fig. 6). The number of the internodes composing this strand is found to be always sixteen. The connecting pieces of each dumb-bell shaped mass are frequently considerably diminished, both in length and in diameter; and very often the enlarged ends of each dumb-bell are drawn out towards the center of the circle. Thus the two neighboring ends form a V-shaped mass with the tip towards the center. This case is very well shown in Figs. 6 and 7. Still another variation of the figure which is met very often, is the intimate union of two dumb-bells. Fig. 10 is an example of this. The small letter (a) of the figure marks the chromosomes where two dumb-bells lie parallel and close together, thus giving the appearance of a single rod. This union of the two dumb-bell shaped masses into a single rod makes it difficult in some cases to count the exact number of chromosomes. In this stage the chromosomes stain most intensely. After the chromosomes combine to form a strand composed of dumb-bell shaped masses, the strand forms a ring, the constituents of which are the V-shaped bodies just described (Fig. 9). This mitotic figure forms the "equatorial plate."

Fig. 9 is a side view of the germinal cells in this stage. Besides the changes of the chromatic substance already mentioned, there are also important changes of the centrosome. Correlated with the changes in the chromatic substance is a migration of one of the central corpuscles to the opposite pole of the nucleus, but in this instance, the migration could not be followed.

From the fragmentary observations of the migration of the centrosome, the present writer believes that one of the central

corpuscles migrates toward the pole opposite to that at which it originates—this migration very probably is correlated with disappearance of the nuclear membrane—and after it reaches its final position, the centrosome divides into two corpuscles, and at the same time the original non-migrating central corpuscles also divides into two, thus forming two central corpuscles at each pole.

The next important event is the formation of the "aster." As figure 9 shows, the somewhat parallel rays formed by minute microsomes connect the two poles; that is, the centrosomes in both poles are connected with these numerous rays. These rays, or "Halbspindelfasern" of HERMANN, are quite different from the rest of the aster rays which also arise from the centrosomes and radiate outwards in all directions. The "Halbspindel" rays are stained intensely with reddish color (HERMANN'S method), while the other rays stain very faintly. Not only is this so, but the former parallel rays are composed entirely of coarser granules than compose the latter. Furthermore, the rays of the latter are directly continuous with the cytoreticulum, while the Halbspindelen rays do not show such continuation, as far as our observation went. The peculiar differences between the "Halbspindelfasern" and the rest of the archoplasm were first reported by LEVI ('98), who studied the mitosis of the nerve cells of the guinea-pig under pathological conditions following mechanical injury, and further LEVI suggested that the "Halbspindelfasern" are directly derived from the acidophile part of the resting nucleolus. Though LEVI has pointed out only the "Halbspindelfasern" as directly derived from nucleolus, the writer's specimens show that a part of the central spindle fiber also has been derived in the same way.

As was mentioned already, the chromosomes in an early spireme stage are surrounded by the nucleolar substance, and this substance, in the next stage, is transformed into parallel rays and extends toward both poles, and finally forms the "Halbspindelfasern." This can easily be followed if one compares the granules which form the spindle with those of the nucleolar sheath of the chromosomes in an early spireme stage.

Fig. 10 is a somewhat polar view of the mitotic figure in the metaphase. The continuous segmented spireme shows, in most cases, a modified dumb-bell shape as was mentioned above. Fig. 7 is a polar view of the equatorial plate drawn in optical section. This figure presents also a modified segmented spireme. Fig. 8 is a cross section of the mitotic figure passing through a plane near the middle of the equatorial plate. The continuous chromosomes were cut in such a way as to present discontinuous V-shaped bodies. The nucleolar substance which thickly surrounds the chromosomes, and also the radial arrangement of the same substance from the center toward the periphery, are distinctly visible in the space surrounded by the chromosomes. This radial arrangement of the nucleolar substance is much more clearly noticeable in Fig. 7.

B. Germinal Cells in the Metaphase. In this phase the chromosomes forming the equatorial plate lose their regular arrangement and lie somewhat irregularly in the equator of the cell-body (Figs. 11 and 12). Meanwhile with this change, each internode of the continuous segmented spireme splits along its long axis. This split, however, stops at the terminal enlarged bulbs; that is, both extremities of the dumb-bell remain undivided. In some cases, these terminal bulbs show deep constrictions along the middle, as can be seen in Fig. 11. Curiously enough, when each internode divides longitudinally, then new enlargements or knobs are produced, one on each side near the middle of each daughter chromosome. This is indistinctly visible in Fig. 11, but plainly shown in Fig. 13. When the chromosomes split longitudinally, the nuclear substance still surrounds the figures, except in very few cases where the nucleolar substance is only visible surrounding each daughter chromosome, but does not fill up the space newly formed by the splitting. After the splitting processes have been completed, the continuously segmented spireme rearranges itself in a manner different from the equatorial plate (Fig. 9) already mentioned; that is, the enlarged portion of the dumb-bell in Fig. 9 lies along the equator of the figure, and the knobs newly formed at the middle point of each daughter chromo-

some, form two rows on either side of the equatorial line and parallel to it. This complicated structure is clearly visible in Figs. 13, 14 and 15.

In this stage, the nucleolar substance, in most of the cases, is accumulated at the two ends of the chromatic figure, although a small quantity of the same substance is also visible surrounding each daughter chromosome (Figs. 14 and 15). Fig. 15 is a semi-diagrammatic drawing to show the real arrangement of the chromosome in this stage, while, in nature, the figure does not appear in such regular way, but always shows more or less modification. Not only so, but the considerable amounts of the nucleolar substance which thickly surround the chromatic figure tend to obscure the details of its arrangement. Fig. 13 is one of the best figures ever observed in the preparation in such a stage. In Fig. 14, the chromatic rods are not shown entirely but only a part. The rest of the structures of the cell-body remain in the same condition in which they were during the last stages of the prophase.

C. Germinal Cells in the Anaphase. The important changes in the chromatic figure in this phase are (1) the transverse splitting of the equatorial plate passing through the enlarged knobs which were primitively extremities of the dumbbell; and (2) the pulling up of the chromatin from the equator toward each pole as a result of the contraction of the "Halbspindelfasern." A very important, as well as interesting point, is the transverse splitting of the entire chromatic figure along the equator. Each of the portions resulting from this division forms a continuous spireme. Although the mitotic figures presented by the nerve cells show certain peculiarities in detail, still they agree exactly with the results obtained by previous investigators in that the chromosomes are divided into two exactly equal halves.

Fig. 16 is a first stage in this phase, and shows the first formation of the central spindle. The central spindle is evidently formed from two different substances. One of these is the linin, which intimately ensheaths the chromosome and the other is the nucleolar substance. The nucleolar substance does

not lose its peculiar staining character, showing always the same intensity of the color. By this character it can easily be distinguished from the other substance. The linin, on the other hand, stains very faintly, but slightly deeper than the cytoplasmic reticulum. According to LEVI, the nucleolar substance (acidophile part), forms only the "Halbspindelfasern," while the central spindle is formed by the linin alone. Our preparations however, show that the latter is not formed by the linin only, but also by the acidophile substance of the nucleolus. When two daughter chromosomes retreat toward opposite poles, then they are connected by delicate filaments composed of both linin and nucleolar substance. In most cases, the nucleolar substance can be detected very easily, since this substance clings to the linin filaments of the central spindles forming thick masses of irregular outline (Figs. 16 and 17). Another important and conspicuous change affects the cell-body which is transformed from the primitive spherical to the oval form.

Following the stage shown in Fig. 16, there is retraction of the two groups of daughter chromosomes toward their own poles (Fig. 18) until they reach two centers of the oval body (Fig. 18). Fig. 18 is a last stage in the anaphase, and presents the beginning of the division of the cell-body. The central spindle is still noticeable in this figure. From this stage on, the central spindle loses its affinity for the coloring reagents employed in this investigation, and at the same time loses the regular parallel arrangement of its filaments which re-form into the cytoreticulum.

D. Germinal Cells in the Telophase. Fig. 19 is one of the newly formed daughter cells, containing half of the chromosomes formed in the dividing cell. When the cell-body has been divided into two, then the regressive processes take place in each daughter chromosome, that is, the chromatic figure which presents an arrangement similar to that of the last stage of the anaphase (Fig. 18) is transformed gradually into that of the nucleus in the resting stage. Fig. 19 shows one of the daughter cells in the earliest stage of the telophase. The

chromatic figure is exactly similar in its principal characters to that of the preceding stage (Figs. 16, 17). In this stage, the nuclear membrane has not yet formed and the nucleolar substance surrounds the figure thickly in an irregular manner. In a far more advanced stage, however, the chromatic figure modifies itself in a remarkable manner, presenting a spiral arrangement along the periphery of the nuclear membrane (Fig. 20). The continuous chromosomes which present a spiral arrangement are constricted at intervals along their course, and divide the spiral line into numerous small segments. The linin could not be distinguished from the nucleolar substance. This stage is shown by Fig. 20. Following changes in the chromatic substance, the nuclear substance re-integrates and produces numerous acidophile granules (Fig. 21). In the next following stage the spiral arrangement of the filament ceases to be visible, but instead of that a reticular arrangement reappears in the nuclear area. The chromatic granules which were constricted from the continuous spiral line, diminish in size to a considerable degree and remain suspended in the reticulum (Figs. 21-23).

The filaments which form the reticulum just mentioned are not the same substance that forms spiral thread (Fig. 20); that is, the nucleolar substance which forms part of the sheath of the chromosome in the preceding stage—not shown in Fig. 20—forms scattered granules which, like the chromatic granules are suspended in the reticulum. The reticulum itself is composed entirely of linin substance. The scattered acidophile particles (nucleoli) are always larger than that of chromatic granules and further they stain a deep blue in the preparations made by HERMANN'S method. This stage is shown in Fig. 21. Large spherical bodies are distinguishable here and there in the figure which represent the scattered acidophile particles of nucleolar substance. These scattered acidophile particles, however, sooner or later are collected (Fig. 22) at one point in the nucleus, generally at the center, although, in many cases, they remain in the scattered form, and do not centralize. Or, very frequently, some of these scattered acidophile particles centralize themselves while the rest of them remain in their original

places (Fig. 23). When the acidophile particles centralize themselves at one place, a circular space is produced. This space, however, appears to be filled by a fluid which also stains a red color after HERMANN'S method. In a further advanced stage, these groups of the acidophile particles, with enclosed liquid, are covered by another chemical substance which stains a blue color by HERMANN'S method. This latter substance seems to be derived from chromatic substance, because, in this stage, the latter material accumulates around the former. The present writer observed also, in most cases, extremely delicate filaments which directly arise from each acidophile particle and further, these filaments fuse together. The significance of these filaments just mentioned will be discussed later on.

IV. Comparison with results of other investigators.

From the preceding descriptions of the mitotic figures, it is clear that during the mitotis of the nerve cell the arrangement of the chromosomes as well as the behavior of the nucleolus is slightly different from that described in other kinds of cells by previous investigators.

FLEMMING[1] first described a peculiar modification of the mitotic figures which is clearly visible in the spermatocytes of the salamander. According to this author, the chromosomes in an early stage of the mitosis do not split entirely, but remain undivided at both ends, thus forming an oblong figure. Each oblong divides again transversely into equal parts and forms U-shaped chromosomes which lie in corresponding positions on each side of the equatorial plane. This U-shaped chromosome is not single, but is formed by two daughter chromosomes which are connected at their tips. To this kind of mitotic figure, FLEMMING has given the name "Heterotypical division or mitosis."

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[1] FLEMMING: Neue Beiträge zur Kenntniss der Zelle: *Arch. f. Mikroscop. Anatomie*, Vol. XXIX, 1887.

In most cases, the U-shaped chromosomes divide again lengthwise, and the four chromosomes of the same shape are produced from a single oblong. This phenomenon is known as "tetrad" formation of the chromosomes.

FLEMMING'S observation was soon confirmed by many investigators and was also extended by HAECKEL on the germinal tract and urogenital cells of Cyclops; by VAN DER STRICHT on the Thysanozoon eggs; by v. KLINCKOWSTRÖM on the Prostheceraeus eggs, etc. In each case, the "tetrad" formation of the chromosome is somewhat similar in its principle to that of the salamander (FLEMMING), although there occur slight morphological differences.

The mitotic figure in the nerve cell may be regarded as one of the modifications of the heterotypical mitosis. A slight difference between the nerve cell and the case of the salamander is the following: The chromosomes in the nerve cell are always continuous throughout the entire course of division, while in the salamander the chromosomes divide into numerous segments which are separated from one another and perform the dividing processes independently. For this reason, the process of the mitosis is more difficult to follow in the nerve cells.

Although typically the chromosomes form a continuous thread, the present writer noticed occasionally somewhat V-shaped chromosomes which had been separated from the continuous group. The writer expects in the near future, to explain these peculiar cases.

V. Morphology of the nucleolus.

As has been already mentioned, the nucleolus of the nerve cell not only plays an important rôle in mitosis, but also presents a peculiar behavior when compared with that of other tissue as described by different authors. This peculiarity, however, depends on the special structure of the nucleolus in the nerve cell. At the later stage of the telophase, the nucleolar substance which intimately surrounds the chromosomes with a thick layer is dissolved and accumulated at certain places in the nucleus forming small spherical masses. These isolated masses in most cases aggregate themselves at the center of the nucleus and form a large group.

The nucleolar granules thus grouped give out one process from each pole. The processes fuse with one another, thus

forming a complete circle of the nucleolar substance (Fig. 22, 23). In the adult stage, more processes are produced. These in turn fuse until there is formed a very complicated network. This latter phase of the nucleolus can easily be seen in the nucleolus of the resting nucleus (Figs. 25, 26, 27).

Sooner or later, after the acidophile particles have been accumulated at one point within the nucleus, the basophile substance as well as the linin surrounds the acidophile particles intimately. These three different substances, which are centralized in the nucleolus, can be beautifully demonstrated in the embryonic tissues which stain properly. This preparation shows us the acidophile substance staining red; the linin a faint red and the chromosomes a deep blue after HERMANN'S method. The nucleolus of the adult nucleus does not show these distinctions clearly, since the basophile substance accumulates about the periphery of the acidophile particles in great quantity and obscures them.

Fig. 25 is a nucleolus taken from a spinal ganglion cell of the gray rat. The material was preserved with the author's sublimate mixture,[1] followed by toluidin blue and erythrosin. In this case the chromatic granules, as well as nucleolus as a whole, stain bluish, while the acidophile particles stain deep blue. The number of the granules is variable even in the same kind of cells.

Fig. 26 is a nucleolus of the same animal preserved with CARNOY'S fluid followed by the same counter-stains. In this, the granules are much more numerous than the former. Fig. 27 is a nucleolus taken from the efferent neurone of Torpedo occidentalis which was preserved with 10% formalin stained with toluidin blue and erythrosin. In this case, we can see the similar granules in the nucleolus. VON LENHOSSÉK[1] distinguished a group of from three to five punctiform strongly

[1] HATAI, S.: The Finer Structure of the Spinal Ganglion Cells in the White Rat. *Journ. Comp. Neurol.*, Vol. XI, No. 1, 1901.

[1] v. LENHOSSÉK: Die feinere Bau des Nervensystems im Lichte neuester Forschungen, *Berlin*, 1895.

stainable particles in the nucleolus which he termed "endonucleoli," but he makes no mention of their significance. Our figures of the internal structure of the nucleolus correspond exactly to that of VON LENHOSSÉK and therefore we identify the acidophile particles with the endonuclei of VON LENHOSSÉK.[1]

The following is a brief sketch of the investigations on the structure and staining reactions of the nucleolus.

FLEMMING[2] regards the nucleolus as composed mostly of the chromatic substance.

ROSIN,[3] who employed BIONDI's stain, maintained that the nucleus as well as nucleolus are neutrophile.

RAMÓN Y CAJAL[4] maintains that the nucleolus is composed entirely of nuclein which, however, was modified in the central portion by long mitotic repose. LEVI[5] has shown that the central part of the nucleolus stains with the acid color, while the periphery of the organ takes a basic color. From the above fact he concluded that the nucleolus of the nerve cells is composed of two entirely different substances; one of these is acidophile and the rest is basophile.

VAN GEHUCHTEN[6] holds the same view as CAJAL, maintaining that the nucleolus is composed entirely of chromatin. BÜHLER[7] described the nucleus of the spinal ganglion cells of

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[1] LEVI was unable to see these granules, which were referred to by V. LENHOSSÉK, in his preparation (See Alcune particularita di struttura del nucleo delle cellule nervose: *Rev. di. patol. nerv. e. ment.*, 1896, f. 4.)

[2] FLEMMING: Bau der Spinalganglienzellen. *Festgabe f. Henle, Bonn*, 1882.

[3] ROSIN: Ueber eine neue Färbungsmethode des gesammten Nervensystems nebst Bemerkungen über Ganglienzellen und Gliazellen. *Neurol. Centralbl. Leipzig*, Bd. xii, 1893.

[4] RAMÓN Y CAJAL: Estructura del protoplasma nervioso. *Rev. tremest. Microg., Madrid*, Vol. I, 1896.

[5] LEVI, G.: Considerazioni sulla structura delle nucleo delle cellule nervose. *Rev. pat. nerv. e ment.*, III, 289-296, 1897.

[6] VAN GEHUCHTEN: L'anatomine fine de la cellule nerveuse, *Neurolog. Centralbl.*, 1897, p. 905, and *Revue Neurologique*, 1897, p. 494.

[7] BÜHLER: Untersuchungen über den Bau der Nesvenzellen. *Verh. der Physik. med. Gesselsch. zu Würzburg*. Bd. XXXI, Nr. 8, '98.

the frog as composed of oxychromatin in very abundant quantity and small amount of basichromatin. He also said that the nucleolus is composed of a substance very similar to the basophile substance of the nucleus, but not identical with it.

TIMOFEEW[1] observed two kinds of nucleoli in the nucleus of the nerve cells of birds, one of which exactly corresponds in its structure to that described by LEVI; while the other is entirely acidophile.

VI. Critique of the observations of previous authors on the nucleolus.

From the above descriptions, we can distinguish two entirely different views concerning the structure and staining reactions of the nucleolus. One of these is represented by LEVI, who regards the nucleolus as composed of two substances, basophile and acidophile. The other is represented by CAJAL and VAN GEHUCHTEN, who believe the nucleolus to be merely a modification of the basophile substance, which has resulted from long mitotic repose.

In the analysis of the cells by histological methods, it is evident that these methods which give the highest degree of differentiation are the ones which should be made the basis of our description. Hence we are justified in following the descriptions of those authors who have been able to obtain the highest degree of differentiation in the cells which they studied and in disregarding the views which have been developed on the basis of negative results.

The results obtained by ROSIN, who states that the nucleolus and nucleus are neutrophile are, no doubt, due to the less differentiation of the stains which were employed by him.

The present writer also questions the conclusions drawn by CAJAL and VAN GEHUCHTEN, because these investigators used only methylen-blue after NISSL. By this method it is absolutely impossible to distinguish the different substances in the

[1] TIMOFEEW: Beobachtungen über d. Bau der Nervenzellen d. Spinalganglien u. d. Sympathicus beim Vogel. *Internat. Monatschr. f. Anat. u. Physiol.*, 1898, H. 9.

nucleus as well as the nucleolus, since methylen-blue, especially after NISSL's technique, colors all the structures a deep blue.

Not only the technique employed, but the age of the animal modifies these staining reactions. The present writer obtained the following results from the resting nucleus of the nerve cells of the adult white rat which had been preserved with CARNOY's fluid followed by toluidin blue and erythrosin: namely, the chromatic granules, as well as nucleolus, were stained bluish-color of equal intensity. Using the germinal cells of the white rat in an embryonic stage, the tissue having been preserved and stained in the same manner as that of the adult animal, it was found that the chromosomes only stain a deep blue, while the nucleolus appeared a deep red.

The present writer agrees with the view of LEVI in so far as he describes the nucleolus as a group of the acidophile particles which are surrounded by the linin. But in addition to the linin, LEVI considers that the basophile substance also surrounds the acidophile particles. To the last statements the present writer cannot agree, because in this case the basophile substance is merely an accumulation, surrounding the nucleolus, and further this hollow sphere formed by the basophile substance is clearly separated from the linin which directly surrounds the acidophile particles. From this, we cannot regard the basophile substance as one of the structural parts of the nucleolus, but simply as lying outside of it.

TOUCHE and DIDE[1] obtained a result similar to that of LEVI, stating that the nucleolus is composed of basophile and acidophile particles. The present writer, however, concludes from their figures that the nucleolus can hardly be regarded as composed of the two substances just mentioned, because, as their figures show, the basophile particles are separate from the acidophile substance. Figs. 1 and 3 of the same paper show separation of the basophile particles from that of the acidophile. The rest of the drawings also show evidently a simple attachment of the basophile particles to the acidophile.

[1] TOUCHE et DIDE: Note sur la structure du noyau et de division amitotique des cellules nerveuse du Cobaye adulte. *Rev. Neurol.*, N. 2, 1901.

CAJAL[1] classified the nucleus of the nerve cells in three large groups. The third type of the nucleus, according to him, is noticeable among the large nerve cells; motor neurones, spinal ganglion cells, cells of PURKINJE, giant pyramidal cells, etc. In these classes of cells, the nucleus is pale, containing the nuclear sap and traversed by a network in which the knobs never carry the chromatic granules. The chromatic granules concentrate themselves surrounding the single voluminous nucleolus and form a perfect spherule. Although his description is not universally applicable, the present writer has observed very often such nuclei as those described by CAJAL in the large somatochrome cells. In these nuclei the basophile granules centralize themselves, surrounding the nucleolus, thus forming compact spherical masses composed of acidophile and basophile substances. This fact offers a very favorable opportunity to determine whether the basophile granules which surround the acidophile substance of the nucleolus can be regarded as one of the structural parts of the latter organ, because in such cases, if LEVI's statement is right, how can we distinguish the basophile particles which were regarded by him as one of the structural parts of the nucleoli from the other part of the basophile? It is impossible to distinguish these, since they are nothing more than degrees of centralization of chromatic granules. The present writer therefore concludes that the nucleolus is a group of the acidophile particles which is surrounded by the linin.

VII. Summary.

In the cerebellar cortex of the foetal cat, a study of the largest germinal cells shows:

1. The germinal cells of the nervous system of the foetal cat present a modified form of the heterotypical mitosis of FLEMMING.

2. The number of the chromosomes represented by internodes of segmented filaments is 16.

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[1] RAMÓN Y CAJAL: Loc. cit.

3. All of the "Halbspindel," as well as a part of central spindle, are derived from the nucleolar substance, the central spindle containing the linin in great abundance.

4. The nucleolus is composed of acidophile particles surrounded by the linin, as brought out by HERMANN'S method.

VIII. Illustrations.

The following are freehand drawings, using oil ¦immersion lens (Obj. L. 16 × Ocular, B & L. 1). The sizes of the cell-bodies as well as those of the nuclei were given in the text.

PLATE XVIII.

Figs. 1-10. Germinal cells in the prophase. Cerebellar cortex of foetal cats; RIPART et PETIT; HERMANN'S, Figs. 3 and 9; GILSON'S fluid; HERMANN'S.

Figs. 11-15. Germinal cells in the metaphase. Cerebellar cortex of foetal cats; RIPART et PETIT; HERMANN'S Fig. 14; GILSON'S fluid; HERMANN'S.

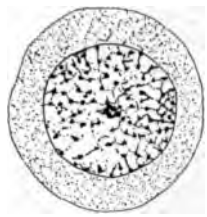
Figs. 16-18. Germinal cells in the anaphase. Cerebellar cortex of foetal cat; GILSON'S fluid, HERMANN'S.

Figs. 19-24. Germinal cells in the telophase. Cerebellar cortex of foetal cats. GILSON'S fluid; HERMANN'S.

Fig. 25. Nucleolus of the spinal ganglion cells of gray rat. The author's sublimate mixture, toluidin blue and erythrosin. 3.2 micra × 3.2 micra.

Fig. 26. Nucleolus of the spinal ganglion cell of the adult white rat. CARNOY'S fluid; toluidin blue and erythrosin. 3.2 micra × 3.2 micra.

Fig. 29. Nucleolus of the efferent neurone of the electric organ of Torpedo occidentalis. 10% formalin; toluidin blue and erythrosin. 5.7 micra × 5.7 micra.



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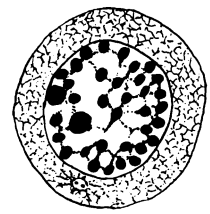

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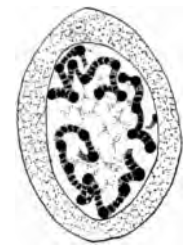

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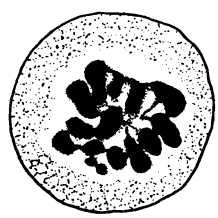

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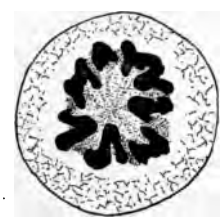

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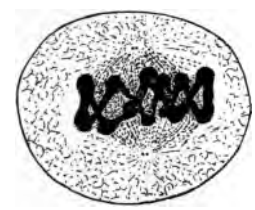

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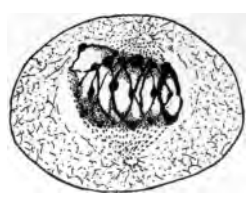

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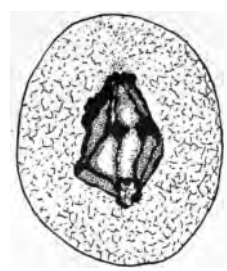

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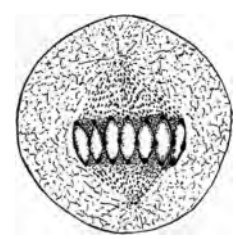

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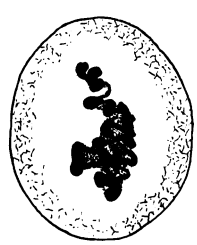

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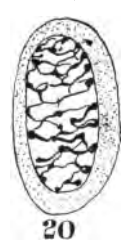

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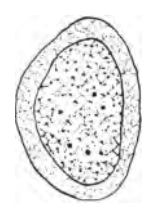

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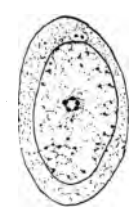

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PLATE XVIII.

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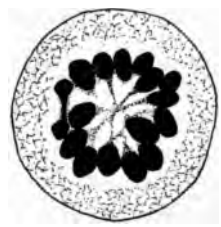

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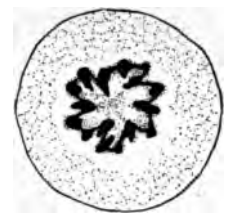

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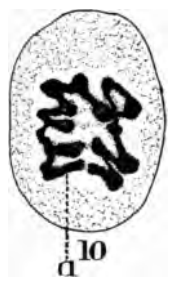

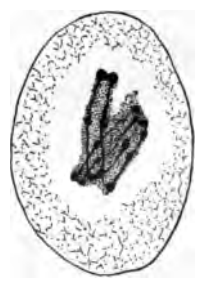

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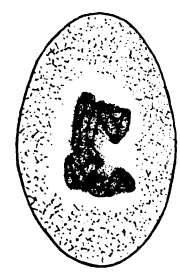

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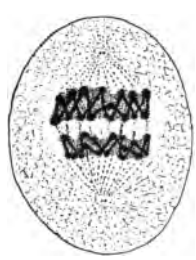

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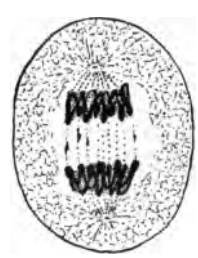

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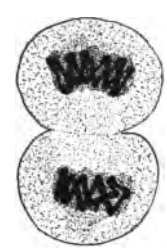

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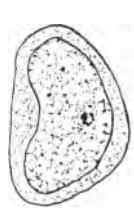

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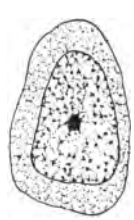

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NUMBER AND SIZE OF THE SPINAL GANGLION CELLS AND DORSAL ROOT FIBERS IN THE WHITE RAT AT DIFFERENT AGES.

By SHINKISHI HATAI.

(*From the Neurological Laboratory of the University of Chicago.*)

I. INTRODUCTION.

The present investigation was undertaken for the following reasons:

(1). In my previous paper (1901, I) on the small spinal ganglion cells in the white rat, these cells were considered to be immature and growing. In that paper, the following statement was made, "From these several observations the writer concludes that the small cells of the spinal ganglion are in a growing state or in a more or less permanently immature condition. The growing fibers (1899) which are found in an adult frog might, therefore, very well be formed by the axones of these latent cell-bodies." If this interpretation was correct, then the number of the small cells should decrease with age, and at the same time, the number of the large cells should increase.

(2). In a second paper (1901, II), the writer made the following suggestion concerning the possibility of the division of the nerve cells in an adult animal, "We can only say, at present, concerning the division problem that the nerve cells in vertebrates, as well as invertebrates, have the centrosome and the sphere, which are regarded as the dynamic centers of the mitotic divisions, and, further, that this centrosome is able to take the first steps of division under certain forms of stimulation, as has been observed by some investigators; but in the normal state the centrosome in an adult cell presents slight morphological differences from that of the embryonic cell, which we interpret as the beginning of degeneration." The writer said further, "In order to give a positive answer to the question (division-problem) above mentioned, it seems to me that the only safe and reliable method consists in counting the nerve cells in the spinal ganglia of a given species of animal at different ages, and thus determining whether there is any increase in their number."

The present investigation consists of a series of enumerations of the spinal ganglion cells and dorsal root fibers, undertaken with a view to settling these questions.

II. MATERIAL AND TECHNIQUE EMPLOYED FOR THE PRESENT INVESTIGATION.

Male rats having a body weight of 10.3, 24.5, 68.5 and 167 grams respectively, were employed. The exact ages of these rats are not known but approximately their weights in grams represents their ages, in days. The ganglia examined were the VI cervical, IV thoracic, and II lumbar. As soon as the dorsal roots with the corresponding ganglia were removed from the cord they were placed on card-board without disturbing the natural length of the nerve roots and preserved in 1% osmic acid solution for 24 hours. After this the specimens were detached from the card-board and washed in running water for 6 hours. Afterward, the specimens were carried through the graded alcohols and embedded in paraffin according to the usual procedure.

For the counting and measurements of the cell-bodies, the sections were cut 12 micra in thickness, while for the nerve fibers 6 micra was found to be a convenient thickness. For the enumeration of the nerve-fibers, the writer employed exclusively the photographic method as used by Dr. HARDESTY (1899) in this laboratory; since, by this method, the enumeration of the nerve fibers is made most accurately and with least fatigue. The countings of the nerve-cells, however, was done with the net under the microscope, using oc. 4 × obj. 8 mm. of ZEISS. Since the large nerve cells (50 micra in diameter), as well as the nuclei (16 micra in diameter) may appear in more than one section, the count was made by enumerating the nucleoli. Fortunately, the presence of multi-nucleoli is very rare in the large cells, though it is not uncommon in the small or smallest cells. Under these conditions, an error in the counting would occur only when one nucleolus was in one section and the other in a second, an arrangement so rare that it may be neglected.

In studying the numerical relations between the cells in the spinal ganglion of several sacral nerves of the rabbit, and the fibers in the corresponding dorsal nerve roots, LEWIN (1896) first counted the cells by enumerating the nucleoli as they appeared in successive sections. This gave him such a large number of cells that he feared that an error had been introduced, owing to the presence of double nucleoli. To correct this, he made another enumeration of cells by the following method. He counted the number of cells which appeared in all of the sections, each section being 10 micra in thickness, and then estimated the average diameter of nerve cells by taking the mean of largest and smallest there present. Employing this average diameter, he then calculated the number of cells. This gave a smaller number than that obtained by counting the nucleoli. The error introduced, however, by employing the average diameter, owing to the variable ratios of large and small cells in any given ganglion, is much greater than that connected with the first and simpler method. For this reason the direct enumeration of the nucleoli, due care being taken to avoid counting double nucleoli, has been used in this case.

In most cases we can determine without any trouble whether in successive sections we are dealing with two parts of the same cells or different cells, and thus the actual error introduced in this way is probably very small. The determination of the small and large cells was made according to the method used in my previous paper on the spinal ganglion cells (1901, I). The class of intermediate cells recognized in that paper was neglected in this study, these cells being classified as large or small according to their diameters. At first, the entire number of the cell-bodies including both large and small, was enumerated. Then the large cells alone were enumerated and their number was deducted from the total, thus giving the number of small cells.

III. ON THE SPINAL GANGLION CELLS.

A. Total Number of the Spinal Ganglion Cells at Different Ages.

The number of the nerve cells in the spinal ganglia has been determined by several investigators:

FREUD ('78), in the spinal ganglia of Petromyzon; HODGE ('88) in the spinal ganglia of the frog; GAULE and LEWIN ('96) in the spinal ganglion of the rabbit; and BÜHLER ('98) in the spinal ganglion of the frog. So far as I am aware, no investigators have ever enumerated the number of the spinal ganglion cells at different ages in the same animal.

The following table shows the total number of the spinal ganglion cells in the three ganglia at different ages:

TABLE I.

Total Number of Cells in the Spinal Ganglia of Male White Rats at Different Ages.

Body Weight Grams.	VI Cervical.	IV Thoracic.	II Lumbar.
10.3	10996	7142	8315
24.5	9793	7068	8200
68.5	11772	7611[1]	9514[1]
167.	12200	7406	9442
Average number	11140	7306	8867

[1] These figures were obtained from a rat having a body-weight of 69 grams and not from the one weighing 68.5 grams, the cervical ganglion of which was alone counted.

As the above table shows, the cervical spinal ganglion contains the greatest number of the cells; the lumbar comes next, while the thoracic contains the smallest number. The most interesting as well as most important point shown by Table I is the approximate constancy of the total number of the ganglion cells during the period chosen. The excess of the number of the cells in the rat of 167 grams over that of 10.3 grams is 10% in cervical and 13% in lumbar, while in the thoracic it is 3.5%. The question at once arises whether this excess means the increase of the number of the cell-bodies with age or whether it is due to individual variation merely. The only argument in favor of interpreting these figures as showing an increase of the number of cells with age is the fact that in the 68.5 and 167 gram rats, the number of cells is greater than in the 10.3 and 24.5 gram rats. Against the idea of the increase is the fact that the 24.5 gram rat shows fewer cells than the 10.3 gram rat, and that in the thoracic and lumbar ganglia the 167 gram rat shows fewer cells than the 69 gram rat. Moreover, there is no indication of cell division in these ganglia. We therefore consider the differences here observed as due to individual variations. The total number of cells is however the sum of two sorts: the small and the large.

BÜHLER ('98) made the following suggestions concerning the small cells: "Es kommt, wie ich mich bei Frosch und Kröte und auch beim Kaninchen überzeugen konnte, physiologischer Weise zum Untergang speciell der grossen Spinalganglienzellen. Die Degeneration verläuft in verschiendenen Formen und allem Anschein nach wenig rapid. Man siet in einem Spinalganglien des Frosches ca. 20-25 untergehende Zellen, beim Kaninchen relativ noch viel weniger. Die verloren gegangenen Zellen müssen ersetzt Werden, und dies geschiet Wahrscheinlich dadurch, das eine der kleinen durch Wachstum ihre Stelle einnimmt. Da nach dem frühesten Jungenstadium eine Vermehrung von Nervenzellen nicht mehr Vorkommt, muss das Spinalganglien, um für die Zeit des Lebens functionsfähig bleiben zu können, in der Anlage genügendes Ersatzmaterial in Gestalt von Reservezellen mitbekommen. Genauere

Untersuchungen hierüber zu machen, bin ich indess noch nicht in der Lage gewesen."

The above interpretation given by BÜHLER concerning the small cells can not be accepted as far as white rats are concerned, for he regarded the small cells as replacing the degenerated large nerve cells; if this were the case, then the total number of the spinal ganglion cells must decrease, but the preceding table shows that the total number is approximately constant.

B. Ratios of Large to Small Cells.

Our assumption that the small cells are in an immature or growing condition, and are more or less transformed into large cells, is proved from the following table which shows that the relative number of the large cells steadily increases.

TABLE II.

Showing the ratios of the large to the small cells.

	Body weight Grams.	Large Cells.	Small Cells.	Total Number.	Ratio L. and S.
IV Cervical	10.3	2526	8470	10996	1:3.4
	24.5	2395	7398	9793	1:3.0
	68.5	3546	8226	11772	1:2.3
	167.0	5080	7120	12200	1:1.4
IV Thoracic	10.3	1557	5585	7142	1:3.5
	24.5	1824	5244	7068	1:2.9
	69.0	2370	5241	7611	1:2.2
	167.0	2902	4404	7406	1:1.5
II Lumbar	10.3	1902	6413	8315	1:3.4
	24.5	2044	6156	8200	1:3.0
	69.0	2934	6580	9514	1:2.2
	167.0	3677	5765	9442	1:1.5

From the table it is clear that the number of the large cells at 167 grams in each region is nearly twice that at 10 grams, showing a ratio of 1:2 approximately. Further, the ratios between the large and small cells in different regions of the animal at the same age is always constant. For instance, the 10.3 gram white rat shows a ratio between the large and small at the three regions of 1:3.4, 1:3.5 and 1:3.4 respectively; in the 24.5 gram rat the ratio is 1:3, 1:2.9 and 1:3 respectively, etc. Finally, the 167 gram rat gives the ratio of 1:1.4 to 1:1.5. From this we can say that each spinal ganglion in the individual at a

given age contains the same proportional number of the small and large cells. The percentage of the small cells in different regions at the same age holds nearly the same proportion. An adult white rat (167 grams) contains, therefore, in each spinal ganglion still 60% of the small cells which were left in an undeveloped condition.

Since the small cells are increasing in diameter from 10.3 grams to 167 grams, the same standard in size cannot be employed in each case. The following table shows the maximum diameters of the small cells at different ages. The small cells, however, cannot be determined by measurement only, for in some cases the cells show evidently the characteristic structures of the large cells in spite of the diameter corresponding to the following table. For this reason, the proper determination of the number of the small cells will not be obtained by measurement only. In this case, therefore, the small cells are those having the diameter less than the diameter of the cell recorded in the table, and at the same time showing the structure characteristic of the small cells as previously determined. (1901, I).

TABLE III.

Showing the Maximum Diameters of the Small Cells at Different Ages.

Regions.	Body-Weights.			
	10.3	24.5	68.5	167
VI Cervical	22.4	24	25.6	28
IV Thoracic	18.8	21	22.8	24
II Lumbar	21.4	22.4	24	27

The diameters of the small cells here given are slightly larger than those given in the table of the previous papers (1) on the spinal ganglion cells in the white rat. This difference is mainly due to the method of selection of the cells for the measurement. In this case two largest small cells from each section (68.3 and 167 grams), and three largest small cells from each section (10.3 and 24.5 grams) were selected for measurement; while in the previous case five largest small cells from each section were measured. Thus, in the latter instance, reducing the average.

IV. THE DORSAL ROOTS.

A. Total Number of the Dorsal Root Fibers at Different Ages.

The number of the fibers of the dorsal roots has been enumerated by several investigators. HOLL ('75) counted the fibers in the two roots and the trunk of three of the lumbar nerves of the frog in order to compare the total number of the fibers in the two roots with that of the corresponding trunks; FREUD ('78) in studying the relation of the dorsal root fibers to the spinal ganglion cells made counts on Petromyzon; STIENON ('80) in studying the relation of the dorsal root fibers to the cells of the spinal ganglion made two counts of the fibers in the two roots and in the trunk; HODGE ('88) counted in the frog the number of fibers of the dorsal roots and the number of the cells in the spinal ganglia of several nerves in order to show the numerical relation between the two; BIRGE ('82) made counts on the dorsal and ventral roots and at the same time the trunk of the several spinal nerves of the frog in order to compare the numbers in these three different regions, as well as to show the relative increase of the fibers and the cells in the anterior horn according to different weights of the frog; GAULE and LEWIN ('96) counted the fibers contained in the two roots and in the trunk and dorsal branches of three of the sacral nerves as well as on the cells of the corresponding spinal ganglia of the rabbit, in order to determine the number in each case; BÜHLER ('98) undertook the problem in order to compare the number of fibers with the number of cells in the spinal ganglion in the frog; HARDESTY ('99) counted in eight spinal nerves, the two roots and trunks, in order to determine the number in each case, and he further extended his studies ('00) on the frog in order to compare the number of fibers in the same regions at different seasons, but in this latter case, the VIth. spinal nerve was used exclusively; DALE ('00) has made similar counts of the fibers in some of the coccygeal, two thoracic and one lumbar nerve of the cat. All investigators (HODGE, GAULE and LEWIN, and BÜHLER) who have compared the number of cells in

the spinal ganglion with the number of fibers in the dorsal nerve root, have found a more or less striking excess of cells in the ganglion.

So far as I am aware, no investigators have ever enumerated the number of the spinal nerve fibers in any mammalian from the period just after birth to maturity. The following table shows the total number of the dorsal root fibers in the roots belonging to to the selected ganglia.

TABLE IV.

Showing the Total Number of the Dorsal Root-Fibers at Different Ages in the White Rat.

Body-Weight Grams.	VI Cervical.	IV Thoracic.	II Lumbar.
10.3	1998	607	723
24.5	2569	863	911
68.5	3683	1420[1]	1317[1]
167.0	4227	1522	1644

From the Table IV it is clear that at any given age the total number of the fibers differs in the three roots of the same animal. It is also shown that the number of the fibers is greatest in the cervical region and that the numbers in the lumbar and thoracic regions follow in order named, except in the case of the 68.5 gram rat, where the number of the fibers is greater in the thoracic than in the lumbar nerve. Taking the number of the fibers in the thoracic as a standard (167 grams), the following ratios are obtained: Thoracic 1, lumbar 1.07, cervical 2.9. Briefly stated, the total number of the dorsal root fibers in the VI cervical nerve at 167 grams is approximately three times as great as in the case of either the thoracic or lumbar at the same age.

The observations of BIRGE ('88) and HARDESTY ('99) on the frog show an excess of the dorsal root fibers in both cervical and lumbar enlargements, and estimates made by STILLING of the area of the cross-section of the dorsal roots in man, show the same thing.

[1] These figures were obtained from a rat having a body-weight of 69 grams and not from the one weighing 68.5 grams, the cervical ganglion of which was alone counted.

The following table will show the relative increase of the dorsal root fibers in the three roots at different ages.

TABLE V.

Showing the Relative Increase of the Fibers at Different Ages.

Body Weight Grams.	VI Cervical.	IV Thoracic.	II Lumbar.
10.3	1.	1.	1.
24.5	1.28	1.42	1.26
68.5	1.84	2.33	1.82
167.	2.11	2.5	2.27

As will be seen from the Table V, the relative increase of the fibers in each region is quite gradual. From the same table it is clear that the fibers in the cervical increase in nearly the same ratio as those of the lumbar nerve, while the fibers of the thoracic, increase more rapidly than the others. This rapid increase of the number in the thoracic nerve will be discussed later on.

B. Ratios Between the Completely Formed and Immature Fibers.

The cross section of the dorsal root at birth, stained with osmic acid presents two kinds of the fibers: one shows clear outline and stains an intense black, while the other shows clear outline and stains with the osmic less intensely, or remains nearly unstained. A careful observation with high magnification, however, reveals to us that the former is surrounded by a sheath which contains an abundance of myelin substance, while the latter possesses a sheath with less myelin substance, which substance sometimes appears as black dots at the side of the sheath. From these facts (WLASSAK '98) the present writer identified the former as the completely formed fibers while the latter he considered as immature. The diameters, however, do not serve to distinguish an immature from the completely formed fibers, for the diameter of the former exceeds, in some cases, that of the latter. Therefore, these distinctions can be determined only by examining the fiber with high magnification, and thus determining whether the sheath contains the full amount of the myelin substance. Using these criteria as the basis of destinc-

tion the present writer enumerated the two kinds of the fibers separately, and obtained the following results, which are represented in Table VI.

TABLE VI.

Showing the Number of Fibers, Completely Formed as well as Immature.

	Body weight grams.	Total number of the fibers.	Fibers completely formed Absolute.	Relative.	Fibers immature.	Percentage of immature fibers.
VI Cervical	10.3	1998	1043	1.	955	48 %
	24 5	2569	2263	2.1	306	12 %
	68.5	3683	3569	3.4	114	3 %
	167	4227	4173	4.	54	1.2%
IV Thoracic	10.3	607	283	1.	424	69%
	24.5	683	497	1.7	366	41%
	68.5	1420	1259	4.4	161	11%
	167	1522	1460	5.	82	5%
II Lumbar	10.3	723	303	1.	420	58%
	24.5	911	678	2.2	233	25%
	38.5	1317	1181	3.8	136	10%
	167	1644	1565	5.1	79	4%

On examining Table VI we notice in the first place the relative increase in the number of mature fibers between 10.3 and 167 grams is less in the cervical region than in the other two. This means that even at 10.3 grams, there is a larger proportion of the mature fibers in the cervical root than in the other two with which it is compared, and the last column in the table giving the percentage of the immature fibers supports this statement. That is, the cervical root of the 10.3 gram rat contains 48% of immature fibers, while that of the thoracic and lumbar contains 69% and 58% respectively. Further, at maturity there is in the cervical root a smaller percentage of the immature fibers than in either of the others, showing that this root is most completely developed. In the same way, if we compare the lumbar with the thoracic root we find the lumbar is always more advanced in its development; that is, it contains smaller percentage of immature fibers. It thus appears that the roots in the cervical and lumbar regions are more completely

developed than in the thoracic. Just why the cervical and lumbar roots should be so far ahead of the thoracic root in this respect is not at the moment readily explained.

The increase in the total number of fibers in the dorsal nerve roots is only to be explained by the outgrowth of nerve fibers from the spinal ganglion. It follows from this explanation that in the immature rat we should expect to find in a given dorsal root a larger number of the fibers near the ganglion than at the entrance of the root into the spinal cord. On this point, HARDESTY ('99) obtained striking results from counting the fibers in the dorsal roots in the frog. He says "The number of fibers in the dorsal roots decreases from the spinal ganglion towards the spinal cord." This observation has been fully confirmed in his most recent paper ('00) on the same subject. On the other hand, DALE ('00), who counted the number of coccygeal dorsal root fibers in the adult cat at different levels was unable to find such numerical differences in the two levels, one near the cord and the other near the ganglion. He says, "The number of the fibers close to the ganglion is the same as the number of fibers several millimeters from it, both proximally and distally, i. e., none of the medullated fibers given off by the ganglion cells end in the nerve roots close to the ganglion." This observation by DALE has been already explained by HARDESTY as follows: "That DALE'S result does not agree with the results previously obtained and here extended is probably due to the fact that the growth of the nervous system of the frog is much slower than that of the mammal. The cat has a fixed period of growth, while the frog, if it does not grow as long as it lives, at least cannot be said not to do so." A similar explanation has been givin by the present writer in his previous paper ('01, I) on the finer structure of the spinal ganglion cells in the white rat.

V. THE RELATIONS OF THE NUMBER OF SPINAL GANGLION CELLS TO THE NUMBER OF DORSAL ROOT FIBERS.

TABLE VII.

Showing the numerical relations between the spinal ganglion cells and the dorsal root fibers.

	Body weights grams.	Total number of cells.	Total number of fibers.	Ratios Fibers and Cells.	Ratios Fibers and large cells.
VI Cervical	10.3	10996	1998	1:5.5	1:1.2
	24.5	9793	2569	1:4.	1: .93
	68.5	11772	3683	1:3.2	1: .97
	167	12200	4227	1:2.7	1:1.1
IV Thoracic	10.3	7142	607	1:11	1:2.5
	24.5	7068	863	1.8.2	1:2.1
	69.	7611	1420	1:5.3	1:1.6
	167	7406	1522	1:4.3	1:1.2
II Lumbar	10.3	8315	723	1:11.5	1:2.6
	24.5	8200	911	1:9.	1:2.2
	69.	9514	1317	1:7.1	1:2.2
	167	9442	1644	1:5.7	1:2.2

On comparing the total number of cells with the total number of fibers in each of three roots, we note first the most important fact that there is always a great excess of nerve cells in the spinal ganglion. Further, it appears that the number of cells corresponding to each fiber diminishes with the growth of the animal. In the case of the cervical nerve of the 167 gram rat thus falls as low as 2.7 cells for each fiber, which indicates that the increasing number of fibers arises by the gradual maturing of the spinal ganglion cells. If we regard the ratio in the case of the thoracic and lumbar nerves we find that the number of cells for each nerve fiber is at all ages somewhat greater for the lumbar nerve than for the thoracic, despite the fact that in previous tables we have found the lumbar nerve more like the cervical than the thoracic. If now, we compare the number of fibers in each case with the number of large cells, we find that in the cervical region at all ages the number of fibers is approximately equal to the number of large cells, while in both the thoracic and lumbar regions, there are on the average, a trifle over two large nerve cells for each fiber. This

would suggest a constitution of the cervical ganglion which was different from that of the other two, since these two have nearly twice the number of the large cells in proportion to the number of dorsal root fibers. It is possible that this difference is to be explained by the presence of DOGIEL's cells ('97) of second type in the ganglia of the thoracic and the lumbar regions, but DOGIEL's statement would hardly support this as a complete explanation, because he distinctly says that the cells in the second type are comparatively few in number, whereas the relation here given would demand a rather large number of the cells.

The excess in the number of the cells over that of the fibers has been reported by several investigators: HODGE, 1888, counted in the frog the number of fibers in the posterior roots and the number of cells in the corresponding spinal ganglia, and found that one afferent fiber of the frog corresponds in these cases to from 2.45 to 3.26 cells. BÜHLER, 1896, found in the dorsal root of the ninth spinal nerve of the frog (Rana esculenta) 680 fibers and in the spinal ganglia about 3500 cells, giving a ratio of 1 to 5; GAULE and LEWIN 1896, found 3173 posterior root fibers in the 32nd. spinal nerve of the rabbit and 20361 in the corresponding spinal ganglia; a ratio of 1:6.4. We see that the excess in the number of spinal ganglion cells has already been observed, but observations here given enlarge our information by showing the excess found in different nerves and at several ages, and, finally, that the ratio diminishes as the animal grows larger.

From the present observations, it is probable that the immature fibers are the processes of some of the large cells, as it will be seen from Table VI that the total number of the large cells exceeds the total number of the fibers. From this fact it is improbable that the immature or smaller fibers in the dorsal root are the processes of the small cells. The excess of the number of the fibers (See Table IV) may be partly explained by the presence of a greater or less number of the apolar cells, in the sense of the early investigators, in the spinal ganglia for the cells of the second type of DOGIEL ('96, '97) (according to

the original describer) are not numerous enough to explain the relations found.

VI. THE SIZE OF THE CELL BODY, THE NUCLEUS AND THE FIBERS AT DIFFERENT AGES.

Twenty of the largest cells with their nuclei, and twenty of the largest fibers, were selected for the measurement in each case. In the case of cell-body and nucleus, three of the largest cells with nuclei from each section, 12μ thick (10.3 and 24.5 grams), and two of the largest cells from each section, 12μ thick (68.5 and 167 grams), were selected for the measurement, while in the case of the fibers, twenty of the largest fibers from one section in each case were measured. The following table shows the results thus obtained.

TABLE VIII.

Showing the mean diameters of the cell-body, nucleus and fibers at different ages.

	Body weight Grams.	Mean diameter of cell-body.	Mean diameter of nucleus.	Mean diameter of fibers.
VI Cervical	10.3	39.5	15 4	7.5
	24.5	42.6	15.9	11.6
	68.5	47.9	16.6	13.3
	167	52.7	17.2	13.9
IV Thoracic	10.3	32.	13.	4.8
	24.5	35.6	13.8	7.1
	68.5	40.3	13.9	8.9
	167	47.2	16.5	11.6
II Lumbar	10.3	33.4	12.5	5.1
	24.5	39.9	14.7	8.
	68.5	43.3	15.8	11.3
	167	51.2	17.5	12.

The cell-body is thus seen to be growing constantly from 10.3 grams to maturity. The cervical spinal ganglion contains the largest cells in each stage, the lumbar comes next in rank, while the thoracic shows the smallest size among the three. In the rats between 68.5 and 167 grams, the mean diameter of the cervical spinal ganglion cells enlarges less than that of the lumbar, while the thoracic and lumbar are nearly alike. The comparison of the cell-body and nucleus shows that the

relative increase of the cytoplasm of the cell-body is much more rapid than that of the nucleus, especially in the last stage of the growth—see Table VIII.

VII. SUMMARY.

1. The total number of the spinal ganglion cells remains approximately constant between 10.3 and 167 grams; though individual variations in the number of the cells in corresponding ganglia exist. It can therefore be stated that this number does not increase or decrease with age—Table I.

2. The cervical spinal ganglia contain the greatest number of cells, while the lumbar and thoracic follow in the order named—Table I.

3. Some of the small cells contained in the spinal ganglia are constantly growing, and in all three ganglia, some of them become large cells—Table II.

4. In all the ganglia the relative number of the large and small cells is nearly the same at the same age—Table II.

5. The cervical dorsal root-nerves contain the greatest number of fibers, while the lumbar and thoracic rank in the order given—Table IV.

6. The relative increase of the number of the fibers in the cervical and lumbar nerves is approximately the same, while that of the thoracic shows a more rapid increase than either—Table V.

7. The dorsal root nerves at different regions in 10.3 gram white rat contain a large percertage of immature fibers giving for the cervical 48%, thoracic 69%, lumbar 58%, while in the mature animal the following percentages are found, 1.2%, 5% and 4% respectively. This indicates the greater numerical completeness of the cervical nerve in both young and the adult. The lumbar nerve follows the cervical, while the thoracic shows the least numerical completeness both in the young and nature—Table VI.

8. The number of the cells in the spinal ganglia is always more than twice that of the fibers in the corresponding dorsal root nerves. The ratios between the fibers and cells at 10.3

grams are as follows: Cervical 1:5.5; thoracic 1:11; lumbar 1:11.5; while in the mature form the ratios are 1:2.7; 1:4.3; 1:5.7 respectively—Table VII.

9. The ratios between the fibers and large cells at maturity are as follows: Cervical 1:1.1; thoracic 1:1.2; lumbar 12:2.—Table VII.

10. Excessive number of cells in the thoracic and lumbar ganglia is possibly to be explained in part by the presence of large numbers of DOGIEL'S cells of second type in these localities. The explanation is, however, not a complete one—Table VII.

11. The mean diameters of the cell-bodies, nuclei and fibers give the highest figures at the cervical region; the lumbar comes next in rank and the thoracic last. The growth of the cytoplasm is always more rapid than that of the nucleus.

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